

HUMAN MUSCLE WEAKNESS AND FATIGUE:
THE EFFECTS OF DISUSE, AGE AND EXERCISE

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ABSTRACT

Weakness and fatigue in the human triceps surae have been assessed objectively by the measurement of absolute force evoked using supramaximal stimulation. The effects of disuse, age and exercise were systematically investigated. Under control conditions the triceps surae of young men were found to generate high maximal tetanic forces, have a mean twitch time to peak tension of 107 msec and did not fatigue readily. This was indicative of a large muscle mass with a predominance of type I (slow twitch) fibres. Muscle temperature manipulation over the range 29.5 to 39.1°C did not affect maximal force generation but had a profound effect on the force and time course of twitch and unfused tetanic responses.

The triceps surae of 70 year old men were found to be slower contracting weaker and yet, paradoxically more fatiguable than, those of young men. These changes may be explained by a slowing of the Ca^{2+} kinetics in the remaining muscle fibres of the elderly and restricted blood supply during intermittent exercise.

Long term immobilisation due to injury caused a substantial reduction in the force generating capacity of the triceps surae and a change in twitch time course which could be explained by selective type I fibre atrophy. In contrast voluntary immobilisation for 2 weeks caused a reduction of maximal voluntary force and a prolongation of the twitch response which could not be accounted for by loss of contractile machinery.

Voluntary dynamic exercise involving concentric contraction of the triceps surae produced small short lasting force decrements. Eccentric contractions caused large long lasting decreases in force particularly at low stimulus frequencies, which were explained by uncoupling of excitation and contraction.

Responses to submaximal stimulation were found to be voltage dependent and did not accurately reflect the response of the whole muscle. The need for supramaximal stimulation in the assessment of weakness and fatigue in the human triceps surae was highlighted.

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1.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Upright posture and the ability to move freely within a chosen environment are heavily dependent in man upon the functional integrity of the muscle of the leg. The large weight bearing muscles comprise two main groups, the quadriceps responsible for the knee extension and the triceps surae concerned with ankle plantar flexion. While these groups greatly influence and may even limit the performance of many activities, reliable assessment of their function has not been made systematically.

Since the primary function of skeletal muscle is to generate force it has been argued that objective assessment of muscle function should be based on the measurement of this criterion (Edwards et al, 1977a; Hosking, 1978;). The simplest method of assessing human muscle function would then appear to be the measurement of voluntary strength using an isometric dynamometer of appropriate design. However Ikai and Steinhaus (1961) showed that the force measured during a maximal voluntary contraction (MVC) of the forearm flexors, is greatly influenced by volition and degree of motivation of the subject. The force developed in a voluntary effort was not simply "a function of the muscle cross sectional area and the physiologic state of its tissues" (sic). These limitations can be overcome by direct electrical stimulation of the motor nerve which supplies the muscle using a supramaximal voltage. This will ensure that all the muscle fibres within the muscle are activated and give rise to a maximal response. The force evoked in this way is

then independent of subject motivation. A further major advantage of this approach is that the evoked responses obtained are comparable to those produced by isolated human and animal muscle preparations designed to investigate specific aspects of the mechanisms underlying the contractile process.

Unfortunately, the human muscles most easily studied in this way are the small muscles of the hand and foot which have readily accessible motor nerves (Merton, 1954; Stephens and Taylor, 1972; Burke et al, 1974; Sica and McComas, 1971) not the large weight bearing muscles of the leg. Nevertheless, attempts have been made to study maximal electrically evoked responses from the quadriceps (Edwards et al 1977a) and the triceps surae (Marsden and Meadows, 1970; Sale et al, 1982) with varying degrees of success. Supramaximal femoral nerve stimulation used to evoke maximal responses from the quadriceps was dismissed by Edwards et al (1977a) as painful and potentially dangerous, with muscle tears, patellar dislocation and bone fracture cited as possible hazards. However, Marsden and Meadows (1970) successfully evoked maximal twitch responses from the triceps surae as well as maximal tetanic responses, though the tetani were confined to frequencies up to 10 Hz, above this frequency stimulation proved impracticable.

An alternative strategy, to minimise discomfort yet still evoke a range of tetanic responses from quadriceps, at a range of stimulus frequencies was developed by Edwards et al (1973). This involved percutaneous stimulation of a portion of the muscle through large pad electrodes applied to the surface of the leg. Responses obtained with this method of stimulation

were said to be representative of the whole muscle in terms of the frequency response relationship and relaxation rate, independent of the stimulus voltage used (Edwards and Newham, 1984). The technique has been successfully used in the analysis of human muscle fatigue. In fatigued quadriceps, relaxation rate and the response of the muscle to tetanic stimuli of certain frequencies have been shown to change markedly, allowing some insight into fatigue mechanisms in man (Edwards, 1972; Edwards, et al, 1977b). However, the major drawback of this approach is that the amount of muscle stimulated on each occasion of measurement and hence the absolute force expected is unknown. Comparison of responses under different measurement conditions can therefore only be made in relative and not absolute terms. This is a serious disadvantage when studying fatigue in weight bearing muscles which must support and move a given body load and therefore must generate finite forces. Inability to generate these forces must impair function yet the degree of this impairment cannot be identified.

If muscle weakness is defined as failure to generate the required or expected force, on first testing or attempted performance (Edwards, 1978) then stimulation of an unknown portion of the whole muscle is clearly of little use in quantifying this weakness since once again the force expected is unknown. This problem becomes further exacerbated when the total muscle mass is changed, as occurs in disuse and with increasing age or when the force generating capacity of muscle changes due to alteration in muscle temperature. The limitations of submaximal stimulation prompted the work

presented in this study. It is the authors that view only by using supramaximal stimulation can responses be obtained which are directly comparable under all physiological conditions. Supramaximal stimulation allows the precise measurement of weakness caused by loss of muscle mass. In the study of disuse atrophy and the measurement of elderly muscle function a maximal evoked response is invaluable since voluntary force can be limited centrally and give a false representation of the degree of muscle wastage. Furthermore, since the time course of a maximal evoked contraction is the resultant of all of the forces contributed by the active muscle fibres it must reflect the overall composition of the muscle. To the authors knowledge the use of supramaximal stimulation in atrophic or elderly triceps surae had not been reported prior to the commencement of this study. Equally the effects of temperature and prior-exercise on the force generating capacity of this postural muscle group had never before been measured. Indeed earlier investigations of exercise related weakness and fatigue using supramaximal stimulation had concentrated on smaller non-weight bearing muscles under abnormal laboratory conditions. While this was beneficial in elucidating fatigue mechanisms in particular, the effects of natural dynamic exercise on the absolute force generated by weight bearing muscle was unknown.

The aims of the study were:-

- (1) to establish a routine and reliable technique for the measurement of force evoked by supramaximal stimulation of the ankle plantar flexors.

- (2) to use this technique to objectively assess the weakness caused by loss of muscle mass or change in the force generating capacity of muscle.
- (3) to quantify weakness and fatigue induced by dynamic exercise in a muscle group which is obliged to support and move a finite load, that of the body.

LITERATURE REVIEW

I. Control of Force generation

The control of force generation during voluntary muscular activity was summarised by Edwards (1978) in the form of a chain (Fig. 1). Those stages up to and including the spinal cord can be designated 'central' and those following as 'peripheral'. This distinction between central and peripheral components of the processes leading to force generation is appropriate in man. It was first investigated by Mosso in the last century (1915) by comparison of voluntary contractions of the finger flexors with those evoked involuntarily by electrical stimulation of the median nerve. The use of evoked contractions overcame the influence of subject motivation and of the psychological factors, which are present in voluntary force generation. These factors were clearly demonstrated by Ikai and Steinhaus (1961) who showed that the expression of human strength was modified, in a predictable fashion, by interventions such as shot, shout and hypnosis.

Central modification of force generation may be effected by alterations in the recruitment of motor units and modulation of motor neurone firing frequency. A motor unit is defined as a single motor neurone and the muscle fibres to which its axon runs (McComas, 1977). It may be classified according to histochemical and contractile characteristics. In a wide range of animals myosin adenosine triphosphatase activity has been shown to relate directly to the intrinsic speed of shortening of the muscle (Bárány, 1967). Based on this finding histochemical

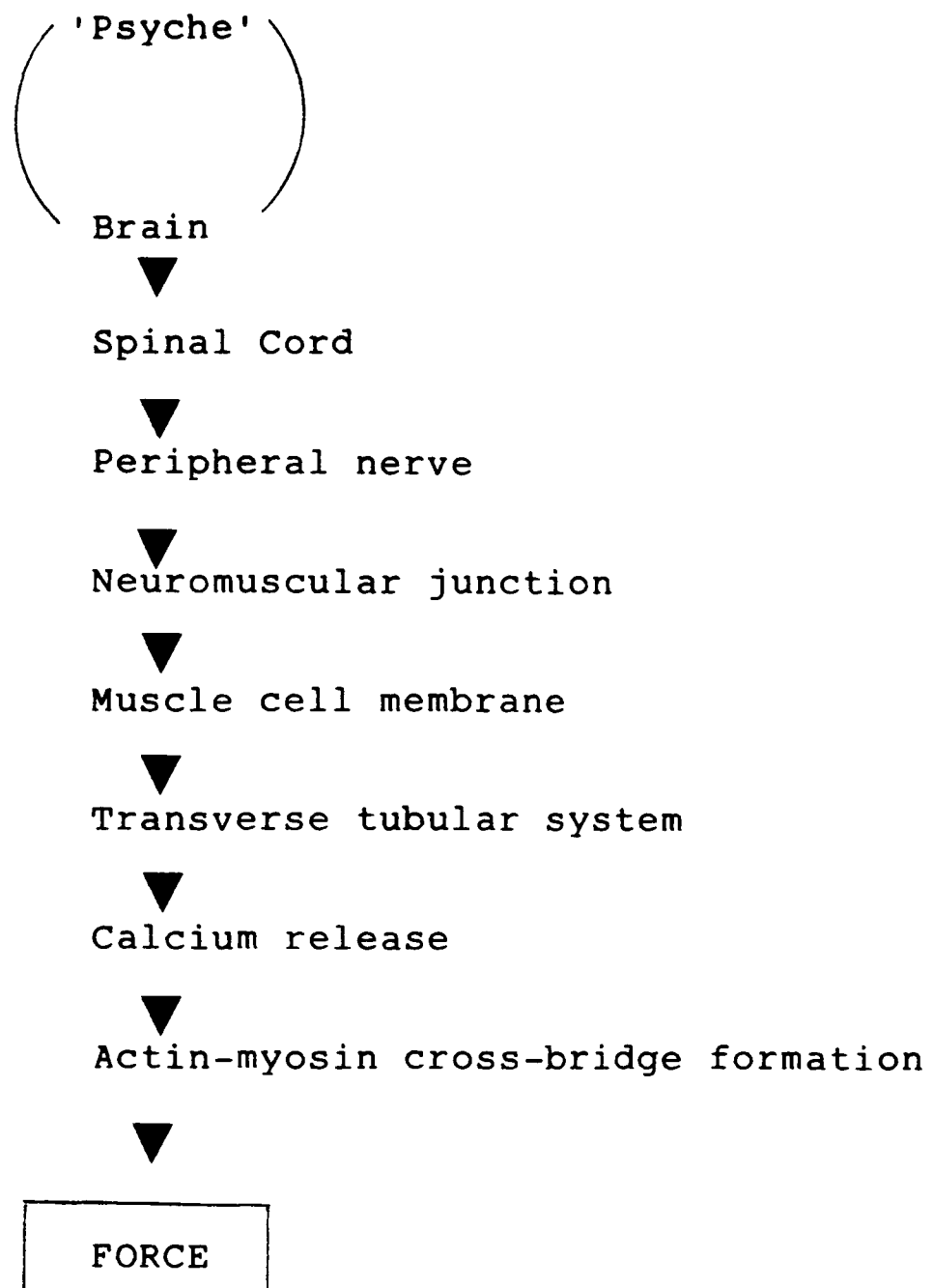


Figure 1.

Voluntary contraction of human skeletal muscle.
(Edwards, 1978).

staining for ATPase activity at pH 9.4 separates slow twitch fibres, designated type I, from fast twitch fibres, designated type II (Engel, 1962). The most commonly used histochemical scheme (Brooke and Kaiser, 1970) further subdivides type II fibres into IIa, IIb and IIc by preincubation for 5 min at acid pH values of 4.5, 4.3 and 3.9 respectively. It is usual to obtain a histochemical profile of the fibre by combination of these findings with staining for oxidative and for glycolytic enzymes. (Buchthal and Schmalbruch, 1980). Type I fibres have high oxidative activity, while type II fibres have high glycolytic activity. Oxidative activity is generally low in type II fibres but is slightly higher in IIa fibres than IIb.

Burke et al, (1973) classified individual cat gastrocnemius motor units on the basis of twitch contraction time and resistance to fatigue in response to stimulation of the motor nerve. Three types of unit could be identified. Type FF units had relatively short twitch contraction times and readily fatigued while FR units had a similar contraction time but were relatively resistant to fatigue. Finally, type S units had relatively long contraction times and were extremely resistant to fatigue. Both FF and FR units stained strongly for myosin ATPase and glycolytic enzyme activity, corresponding to type II fibres histochemically, while the oxidative enzyme activity of the FF units was low and that of the FR units was termed intermediate. Type S units stained weakly for myosin ATPase and glycolytic enzymes but strongly for oxidative enzymes, corresponding to type I fibres. These results supported the view that the histochemical characteristics of muscle fibres

were meaningfully related to their physiological properties. Using controlled intramuscular microstimulation Garnett et al (1979), were able to identify these same motor unit types in human gastrocnemius on the basis of their mechanical properties. Type S units were slow, small and fatigue resistant. FR units were fast, intermediate in size and fatigue resistant while FF units were fast, large and fatiguable. After glycogen depletion to identify the muscle fibres from the stimulated motor unit, biopsy samples showed the type S and FF units to be composed of muscle fibres histochemically classified as type I and type IIb respectively.

Freund (1983) states that experiments on single motor units recorded from human hand and forearm muscles during voluntary contractions support the hypothesis, derived from animal experiments by Henneman (1957) that their recruitment and firing properties, the contractile force they produce, their metabolic pattern, and their fatiguability are determined by their size. Under most conditions, recruitment of human motor units has been shown to progress in an orderly fashion, independent of the speed of movement or isometric contraction (Desmedt and Goddaux, 1977). The level of force at which a motor unit is recruited, its threshold force of recruitment, does however decrease as the rate of rise of contractile force is increased. Spike triggered averaging techniques have shown that human motor units are normally recruited in order of their increasing contraction strength, diminishing fatigue resistance and increasing contractile speed (Milner-Brown et al 1973; Stephens and Usherwood, 1977). This is supported by the histochemical

studies of Gollnick et al, (1974a and 1974b). They found that following low force contractions of the quadriceps, biopsy samples showed type I fibres were depleted of glycogen while at higher forces the type II fibres were also depleted. This was true of both static and dynamic exercise indicating that during low force contractions there was preferential use of type I fibres while the type II fibres were reserved for greater effort. The stereotyped sequence of activation can be modified by cutaneous afferent input. Stephens et al (1978) showed, reversal of recruitment order in motor units of the human first dorsal interosseous (FDI) produced by cutaneous electrical stimulation of the digits

Motor neurone firing frequencies range from 6-8/s up to 120/s in man. However, the highest rates can only be achieved during the rising phase of rapid ballistic movements and cannot be sustained for more than 100 msec (Freund, 1983). The working range of voluntary activated human motor neurones extends from 6-8 to 20-35/s, during steady contractions (Bigland and Lippold, 1954; Milner-Brown et al, 1973). This range matches the range of partial fusion of the corresponding muscle fibres as shown by stimulation of the whole muscle (Marsden and Meadows, 1970; Edwards et al, 1977a) and by microstimulation of single human motor units (Garnet et al, 1979).

II. Muscle Weakness

As previously stated weakness can be defined as a failure to generate the required or expected force on first testing or attempted performance (Edwards 1978). Clearly weakness could be caused centrally by a failure to recruit motor units or to drive them at the appropriate frequency as described earlier. This may be due to a lack of motivation, psychological limitation or neurological disorder. Muscle could also fail to generate force by the peripheral mechanisms shown schematically in Fig. 2. Electromechanical activation may fail in certain clinical conditions such as myasthenia gravis but impaired energy supply does not appear to be the cause of clinical weakness (Edwards, 1978).

Loss of contractile machinery in otherwise healthy individuals commonly occurs in disuse atrophy caused by limb immobilisation and in old age. Due to the arrangement of contractile proteins within muscle fibres this loss could be as a result of decreased fibre number, fibre area or a combination of both.

(a) Immobilisation

In animal studies the evidence for a reduction in fibre number following immobilisation is contentious. Booth and Kelso (1973) reported a decreased total fibre number in rat soleus after 4 weeks plaster cast immobilisation of the hind limb. However, Cardenas et al (1977) in a similar study found no change in fibre number and claimed that Booth and Kelso were in error. Cardenas et al counted fibres in a small portion of the

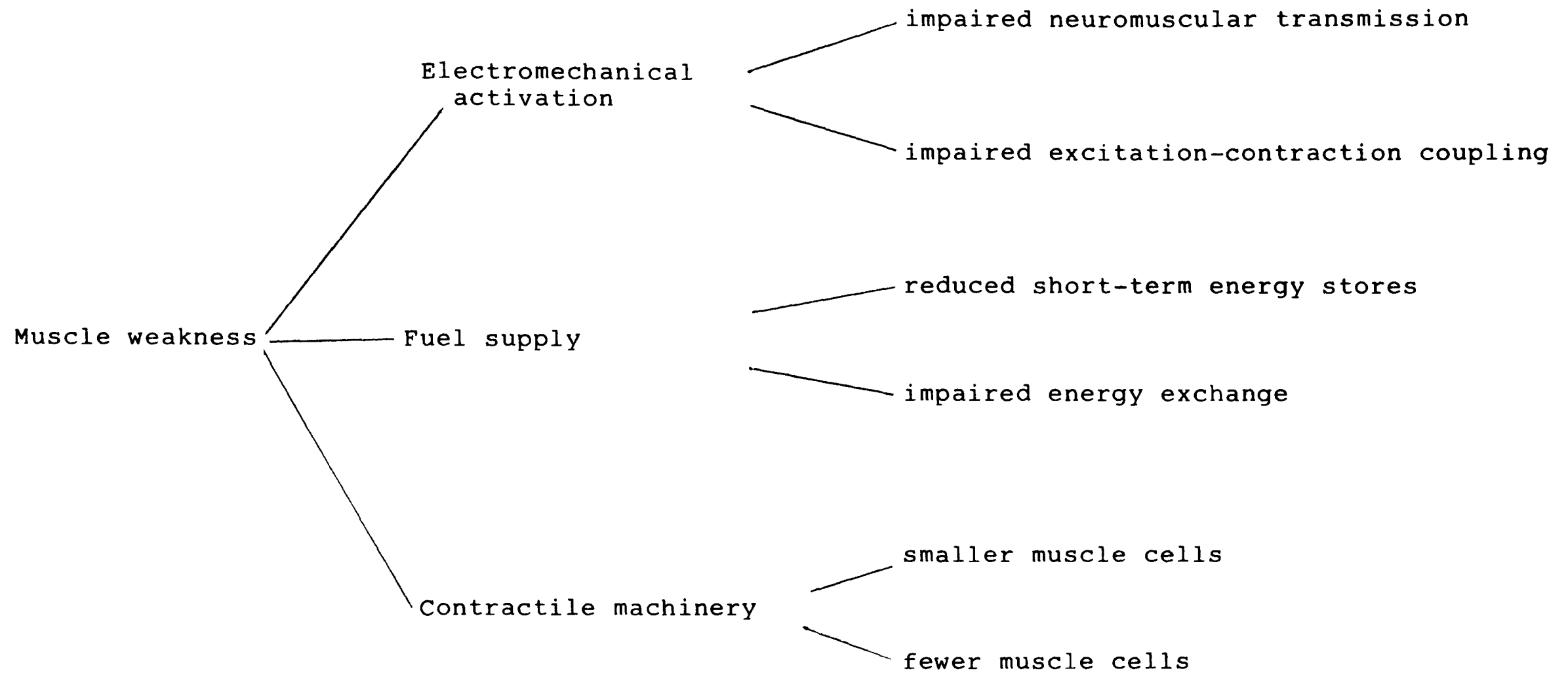


Figure 2.

Practical scheme for the analysis of muscle weakness (Edwards, 1978).

total muscle cross section and then calculated total fibre number. They repeated this in numerous cross sections along the length of the muscle. Since soleus muscle fibres do not run the length of the muscle they claimed that by using a single cross section in the belly of the muscle, Booth and Kelso did not count all of the fibres. Boyes and Johnson, (1979) found no reduction in total fibre number in rat vastus intermedius while Mayer et al (1981) inferred similar findings in single motor units of cat medial gastrocnemius after joint fixation induced atrophy. This was based on the finding of comparable decreases in fibre area and tetanic tension for each motor unit type.

Decreased fibre area after immobilisation has been well documented, however the degree of fibre atrophy appears specific to the fibre type and to its location in different muscles or muscle regions. In animal experiments it is possible to weight excised muscles to determine muscle mass. Herbison et al, (1978) found equal percentage weight losses in the gastrocnemius, soleus and plantaris of the rat after 6 weeks plaster cast immobilisation. Atrophy of type I and type II fibres was equal in the soleus but in the plantaris was greatest in type II fibres. Maier et al (1976) studied the guinea pig hind limb and found that after 4 weeks of immobilisation in a plaster cast the decrease of fibre area in the gastrocnemius was greatest in type I fibres, while the cross sectional area of this same fibre type in the soleus was further reduced. Preferential atrophy of type I fibres was also shown in the cat gastrocnemius by Mayer et al (1981) and in the bush baby plantaris by Edgerton et al (1975b). A reduction in the percentage of fibres staining as type I was

shown in the study of Boyes and Johnson (1979) which in the absence of a decrease in total fibre number was taken as evidence of fibre conversion from type I to type II. Also Maier et al (1976) reported the appearance of type II fibres in the normally 100% type I fibre guinea pig soleus after immobilisation.

By definition reduced type I fibre percentage and selective type I fibre atrophy must increase the relative area of type II fibres. Booth and Kelso claimed that this relative increase in type II area was responsible for the increased speed of contraction of the rat soleus after immobilisation, a finding also reported by Fischbach and Robbins (1969) and by Maier et al (1976) in the Guinea pig soleus. However in a study of single motor units of the cat gastrocnemius after immobilisation Mayer et al (1981) found that all the motor unit types showed a trend towards decreased twitch contraction time especially the slowest units (type S). Thus the change in whole muscle contraction time seen in the earlier studies may not be due solely to change in relative fibre areas.

In man decreased fibre area following immobilisation because of leg fracture has been shown by Sargeant et al (1977). Biopsy samples from the vastus lateralis of the injured limb showed a mean decrease in fibre area of 42% relative to the uninjured limb. The fall in type I fibre area averaged 46% while that of the type II fibres was only 37%. Haggmark et al (1981) reported selective type I fibre atrophy in the vastus lateralis of patients following knee surgery and immobilisation for 5 weeks. This was also observed by Edström (1970) in the

vastus medialis of patients with chronic anterior cruciate ligament injury. Selective type I atrophy was found in the soleus by Häggmark and Eriksson (1979) after surgical repair of the Achilles tendon and 6 weeks immobilisation.

The occurrence of each fibre type was found to be unchanged in the studies of Sargeant et al (1977) and Häggmark and Eriksson (1979). Assuming no decline in total fibre number this would indicate that fibre conversion had not taken place and that the total type I fibre area had declined in relation to type II fibre area. Evidence for the assumption that fibre number does not change after injury and immobilisation comes from the work of Young et al (1982). They used the ratio of quadriceps cross sectional area, measured by ultrasound, to mean fibre area, as an index of the total number of muscle fibres present in the muscle. Comparison of the values for injured and uninjured limbs by linear regression revealed a relationship statistically indistinguishable from the line of unity, therefore indicating equal fibre number in both limbs. Changes in relative fibre area may be expected to change muscle contractile properties but with the exception of voluntary strength (MacDougall et al, 1977) no measurements of the effects of immobilisation on the isometric contractile properties of human muscle had been made prior to the present study.

(b) Age

Studies of ageing animals have shown that the decrease of muscle mass is due to decrease both in number (Gutmann and Hanzliková, 1966; Rowe, 1969; Tauchi et al, 1971) and diameter

(Rowe 1969; Tuček and Gutmann, 1973) of the fibres. The changes are not uniform in muscles of different function. Tauchi et al, (1971) reported a decreased diameter in the fast fibres of the senescent rat tibialis anterior but a decrease in the number alone of the slow fibres. A more rapid decline in the number of muscle fibres in the slow twitch soleus as compared with the fast EDL was reported by Tuček and Gutmann, (1973). Also in the rat soleus preferential type II atrophy was seen by Bass et al (1975) in 28-36 month old animals. By contrast little if any decrease in number and diameter of diaphragm muscle fibres was found by Tuček and Gutmann, (1973).

In man a decline in adult voluntary hand grip and lumbar strength with increasing age above 30 years was shown by Quetelet in the last century (1842). More recent studies have found voluntary strength to be better maintained in modern times, showing a slow or imperceptible decrease from its peak by the 5th decade of life, followed by an accelerated decline (Larsson, 1982).

Where different muscle groups have been compared within the same study there is evidence that voluntary strength declines in later life, while following the same overall time course are greatest in the leg muscles (Asmussen and Heebøll-Nielsen, 1962; Simonson, 1947; McDonagh et al, 1984). Quadriceps strength and morphology in relation to age have been extensively studied in an attempt to discover the mechanisms responsible for the changes seen. It is generally agreed that there is a decrease in the area of type II fibres in the elderly quadriceps but exactly when the decrease begins is not certain. Larsson (1978)

reported a decrease in type II fibre area with increasing age in groups of subjects aged 20-29 up to 60-65 years. This decline in fibre area was associated with but not solely responsible for the decline in voluntary strength seen over the age range. However, Aniansson et al (1981) found no reduction in type II area until age 70 years and Grimby et al (1982) reported only minor decreases in the fibre area of 78-81 year olds. This they argue means that the loss of muscle mass seen in middle and old age (Allen, 1960; Tzankoff and Norris, 1977) must be due to a reduced number of muscle fibres. The same explanation was also used by Larsson (1978) to account for the partial correlation of strength and fibre area.

A reduction in the total number of muscle fibres in the quadriceps was shown by Lexell et al (1983) when comparing 70-73 year old men and 19-37 year old men at autopsy. The younger muscles contained around 500,000 fibres on average while the elderly muscles had over 100,000 fewer fibres.

Clearly major changes occur in the postural muscles of the elderly. However the effects of reduced fibre number and the possible selective loss of type II fibre area on the maximal evoked contractile characteristics of the elderly Triceps Surae are unknown.

III. Fatigue

Most definitions of muscle fatigue include within them the terms failure to maintain, or failure to sustain force in order to distinguish the definition of fatigue from the definition of weakness, which also makes reference to failure of force generation. Mutch and Bannister (1983) however, defined fatigue generally as a decreased ability of an organism or one of its parts to respond or function because of prolonged exertion or repeated stimulation. In more specific terms Asmussen (1979) defined muscle fatigue as a transient decrease in performance capacity of muscles when they have been active for a certain time, usually evidenced by a failure to maintain or develop a certain expected force or power. This has been further refined by Edwards (1981) so that the definition of muscle fatigue most commonly in use is, failure to maintain the required or expected force. Fatigue in these terms can be thought of as a specific form of weakness and, as with weakness, the failure of force generation could arise as a result of central or peripheral processes, a fact first appreciated by Mosso (1915) when comparing voluntary and evoked contractions in his finger 'ergograph'.

Central fatigue was inferred by Asmussen and Mazin (1978) to explain the positive effect of a diverting activity such as opening the eyes after failure of repetitive voluntary contractions with eyes closed. More direct evidence was provided by Bigland-Ritchie et al (1978) when they compared the force produced by voluntary and stimulated contractions of the quadriceps under conditions of local ischaemia. Well motivated subjects attempted to sustain a maximal effort for 60 s during

which force fell to about 30% of the initial level. At 15 s intervals the voluntary contraction was interrupted by a brief period of electrical stimulation. Over the first 30 s both voluntary and stimulated contractions fell to a similar extent indicating that fatigue during this phase could only have arisen by failure at or distal to the neuromuscular junction. However, in 5 out of 9 subjects during the latter part of the sustained contraction the force evoked by stimulation was better maintained than the voluntary force. This indicated that in these subjects loss of neural drive contributed to the force loss. Earlier studies by Merton (1954) and Ikai et al (1967) (see Asmussen, 1979) on the adductor pollicis produced conflicting results. Merton found no increment in force could be produced by electrical stimulation during a sustained MVC, indicating that the site of fatigue was peripheral. Ikai et al (1967) found otherwise, more force could be generated during evoked contractions than during repeated maximal voluntary efforts and furthermore the drop in force was smaller in the evoked than the voluntary efforts, indicating some central fatigue. This study differed from those of Merton and Bigland-Ritchie et al in that it used brief maximal efforts repeated one per second rather than a sustained maximal effort. This raises two factors which must be taken into account when comparing these different approaches. Firstly, Ikai et al (1967) show that stimulated forces exceed voluntary forces even five seconds after the start of the experiment while Merton found stimulated and voluntary force to be equal. This suggests that the voluntary efforts recorded by Ikai et al were not maximal in the first instance,

possibly due to the very short time allowed for contraction and relaxation in each cycle of 1 second. Secondly, relaxation between the short contractions would allow blood flow to the muscle (Barcroft and Millen, 1939) which was not possible in the studies of Merton and Bigland-Ritchie due to inflation of a pressure cuff around the arm. Ikai et al may in this way have delayed fatigue of the peripheral contractile mechanism (Asmussen, 1979) and indeed the fall in force seen in evoked contractions was only 32% compared to the 55% fall seen by Bigland-Ritchie et al. It is now generally believed that central fatigue is not a limiting factor for force generation, at least during sustained isometric voluntary contractions (Bigland-Ritchie, 1981).

Theoretically, peripheral fatigue may be due to any or all of presynaptic failure, synaptic failure, muscle fibre action potential failure, excitation contraction uncoupling or failure of the contractile machinery. Presynaptic failure due to an inability of the nerve impulse to invade the axon terminal was found by Krnjević and Miledi (1958) in rat diaphragm. When recording end-plate potentials intracellularly in two fibres belonging to the same motor unit, they found that on some occasions a normal end-plate potential in one fibre was not associated with any detectable response in the other. This defect was best explained by failure of the impulse to invade the axon terminal. However, these observations were made in vitro and were very sensitive to anoxia. In the single motor unit studies of Burke et al (1973) on cat gastrocnemius and Garnett et al (1979) on human gastrocnemius the blood supply was

intact. These studies found no evidence of a decline in motor unit action potential amplitude which indicates unimpaired excitation and the absence of fibre drop out. This was despite prolonged periods of stimulation lasting up to 1 hour and involving 47,000 stimuli in the cat or up to 2.5 hours and 72,000 stimuli in man. Synaptic failure is equally ruled out by these findings as is failure of the muscle fibre action potential. It must be stated that the experimental protocol used by Burke et al and Garnett et al involved the use of intermittent stimulation deliberately to avoid these problems, under other experimental conditions, such as a sustained maximal contraction there is evidence for and against excitation failure.

In the study of Merton (1954) maintenance of the evoked action potential during a sustained isometric MVC of the adductor pollicis indicated that transmission and excitation was normal. Naess and Storm-Mathisen (1955) on the other hand found a decrease in action potential amplitude during sustained isotonic contractions of the same muscle, though with such a contraction the initial level of activation cannot be as high as in an isometric contraction and this may confound the interpretation of the results. Stephens and Taylor (1972) also found that the size of an evoked action potential was reduced during a maintained isometric MVC of the first dorsal interosseus, the major part of which occurred within 1 minute. Smoothed rectified electromyogram (s.r.e.) recorded during this phase fell linearly with force which on the basis of the reduced action potential was interpreted as failure of the neuromuscular junction, rather than central drive. Later force declined

faster than s.r.e. which was seen as indicative of contractile element fatigue in the low threshold motor units still active. This conclusion was challenged by Bigland-Ritchie et al (1978) who accounted for the parallel decline in s.r.e. and force, seen in their study of sustained MVC's in the quadriceps, by the mechanism of central fatigue. They based this on the observation that evoked contractions were better maintained than voluntary contractions in some of a group of subjects. In other subjects, contrary to Stephens and Taylor, they found that the s.r.e. remained almost constant throughout the contraction while the force declined, indicating maintained central drive and no neuromuscular junction failure. Finally, they questioned the influence of factors such as firing frequency and synchronisation on the surface recorded signal and stated that in their view this signal provided no evidence for neuromuscular junction failure as the limiting factor determining loss of force in the voluntarily activated quadriceps. In a subsequent study on adductor pollicis (Bigland-Ritchie et al, 1979) the total area of the evoked surface action potential (SAP) was shown to increase slightly during sustained MVC's, a finding totally at variance with that of Stephens and Taylor. This it was suggested was due to the method used by Stephens and Taylor to measure SAP size. The criticism of their method was that only the portion of the SAP above the isoelectric line was measured, and this took no account of a change in the shape of the response caused by slowing of conduction in the muscle fibre. It is believed that this slowing is due to accumulation of K⁺ in the extracellular spaces of the muscle (Bigland-Ritchie

et al, 1979; Jones, 1981).

In the most direct demonstration of maintained muscle excitation during voluntary fatigue in man Merton et al (1981) showed that action potentials in the adductor pollicis evoked by stimulation of the motor cortex were unaffected. Thus, indicating that the whole motor pathway conducted normally. Twitches, evoked by direct stimulation of the muscle with massive pulses of up to 2,400 volts, were however greatly reduced showing that during a sustained MVC in this muscle it is the muscle fibres that fail. A similar conclusion was reached by Petrofsky and Lind (1979) following experiments on cats which involved computer controlled sequential stimulation of ventral root bundles and anodal block to mimic the recruitment and firing pattern believed to occur in voluntary effort. They found that during sustained contractions of between 3% and 100% of maximum isometric tension, endurance did not appear limited by transmission failure in plantaris, gastrocnemius or soleus unless high stimulation frequencies of over 80-100 Hz were used. As already discussed such values and higher have been reported in human studies (Marsden et al, 1971) but only for brief periods during maximal effort. From this evidence then it would seem that failure of excitation is unlikely to play a great part in fatigue associated with most natural activities undertaken by man since firing frequencies are rarely high enough for long enough to cause it (Bigland-Ritchie, 1981). However, the matter cannot be considered settled as Hultmann and Sjöholm (1983) showed that low frequency submaximal stimulation of quadriceps at 20 Hz caused the amplitude of the surface

recorded action potential (SAP) to fall in parallel with force during a 75 s continuous tetanus. Interestingly, during recovery SAP amplitude quickly returned to normal while force remained low. This indicated that excitation failure was not the sole cause of fatigue and suggested that excitation-contraction (E-C) uncoupling had occurred.

The failure of force generated by low stimulus frequencies due to E-C uncoupling has been demonstrated in animal (Grabowski et al, 1972) and human muscle (Edwards et al 1977b) after both stimulated and voluntary contractions. It has also been shown to be long lasting in man and particularly prevalent under ischaemic conditions (Edwards et al, 1977b; Edwards, 1981). The principal reasons for the belief that this form of fatigue is due to E-C uncoupling in man are a maintained SAP and a normal response to high frequency stimulation. Furthermore in animal studies caffeine or other methylxanthenes restore normal force indicating that the contractile machinery is also unaffected (Grabowski, et al, 1972; Jones et al, 1982).

Low frequency fatigue results in a shift of the steep portion of the frequency response curve of a muscle towards higher stimulus frequencies (Edwards et al, 1977b). Since firing rates for many normal activities involving submaximal contractions are believed to lie in the low range of 10-30 Hz (Grimby and Hannerz, 1977; Freund, 1983) a considerable loss of voluntary force would be expected if normal firing was to continue during low frequency fatigue.

It has been shown in animal studies that fibres with a high oxidative enzyme activity are most resistant to low frequency

fatigue (Kugelberg and Lindegren, 1979) but its prevalence in human muscle is less well known. Edwards et al (1977b) found low frequency fatigue of the adductor pollicis and quadriceps after rather unnatural, repeated sustained isometric contractions made under ischaemic conditions. Similarly, Moxham et al (1981) showed low frequency fatigue of the sternomastoid after loaded breathing and sustained maximum voluntary ventilation. In this study the authors also demonstrated the phenomenon in the diaphragm, using the same fatiguing regime, despite the fact that the diaphragm is composed of fibres with high oxidative enzyme activity (Lieberman et al, 1973). Clearly, in man such a metabolic profile does not totally protect against low frequency fatigue. It should be noted that of the human muscles mentioned above only the adductor pollicis was stimulated supramaximally allowing direct measurement of the fatigue induced.

The occurrence of low frequency fatigue in other muscles with high oxidative enzyme activity, following natural activities is little known, though it has been shown in the quadriceps of some subjects with unreported fibre composition, after cycling and stepping (Edwards, 1977b). This is particularly relevant in the large weight bearing muscles of the lower leg, primarily Gastrocnemius and Soleus, which are known to possess a high percentage of type I fibres (Edgerton et al, 1975a; Johnson et al, 1973). These muscles contribute 90% of plantar flexor force (Murray et al, 1976) and are heavily involved in the maintenance of posture and locomotion where repetitive low frequency firing would be expected on the basis of animal and human studies (Eccles et al, 1958; Grimby and

Hannerz, 1977; Freund, 1983).

A final characteristic of fatigue; which can be ascribed to failure within the muscle fibre, is a slowing of relaxation rate as fatigue progresses. This was first observed by Marey in the last century when studying frog muscle (in Mosso, 1915) and has since been reported in animal and human muscle (Edwards et al, 1972; 1975). The mechanisms responsible for the time course of relaxation are not fully understood but two main possibilities for the rate limiting step in relaxation have been considered, Ca^{2+} reuptake by the sarcoplasmic reticulum (S.R.) (Sandow, 1965; 1970; Briggs et al, 1977; Dawson et al, 1980) and cross bridge dissociation (Edwards et al, 1975).

For the latter mechanism to control relaxation, slowing of relaxation must involve a reduction in the turnover of cross bridges and a decrease in ATP turnover. Experimentally there is evidence both for and against a reduction in ATP turnover during fatigue. Edwards et al (1975) found such a change in fatigued mouse muscle using conventional biochemical techniques while Dawson et al (1980) did not in frog muscle using nuclear magnetic resonance techniques. Evidence for Ca^{2+} accumulation by the S.R. as the rate limiting step comes indirectly from the association of relative Ca^{2+} uptake capabilities of the S.R. from fast and slow muscles. (Sandow 1970; Brody 1976; Briggs et al, 1977). It is also the mechanism favoured by Dawson et al (1980) after failing to demonstrate a change in cross bridge cycling rate in frog muscle.

The distinction between central and peripheral components of force generation involved in muscle weakness and fatigue has

been made. From animal models and study of human hand muscles the importance of supramaximal stimulation in the assessment of muscle function in man is clear. This thesis examines the maximal evoked mechanical properties of large weight bearing muscles with reference to use, disuse and age.

CHAPTER II

Human Muscle Function

1 - Measurement of the Isometric Contractile Properties of the Human Triceps Surae.

Studies of the in vivo contractile properties of the human triceps surae are few and the methods used disparate. Buller et al (1959) measured the time course of twitch contractions of fibre groups in gastrocnemius and soleus indirectly. A saline filled needle was inserted into the belly of the muscle and used to both stimulate the fibres near it and record the associated pressure changes caused. The time course of these pressure changes was shown to approximate to the time course of the twitch response measured by conventional strain gauge methods in cat muscle. Another form of indirect measurement was used by McComas and Thomas (1968) who used weak electrical stimulation of the motor nerve to the lateral gastrocnemius to produce maximal H reflexes. Force transduction in this case was by a stiff titanate bender element which had a plastic stylus attached to it. The stylus was pressed into the skin over the muscle belly. Again the system was validated by comparison with direct measurements in rabbit soleus, where time to peak tension was found to be identical using both systems but half relaxation time was up to 10% faster with the indirect method Marsden and Meadows (1970) evoked maximal twitch and unfused 10Hz tetanic contractions of the triceps surae by stimulation of the medial popliteal nerve. Force was measured at the ball of the foot by a strain gauge fixed to a foot plate with heel lift prevented by a 20 kg weight placed on the knee of the seated subject. Higher frequency stimulation proved to be impracticable, presumably because of the discomfort and inadequate downforce at the knee,

therefore the maximal tetanic tension could not be measured.

Motor units in medial gastrocnemius were studied by Garnett et al (1979) using controlled intramuscular microstimulation to evoke twitch and tetanic contractions. The small forces developed were recorded as torque about the ankle joint by a sensitive torque meter.

The much larger forces developed by maximal voluntary and evoked tetanic contractions require the use of a more robust dynamometer such as the one described by Lippold (1952) when studying the relationship between integrated action potentials and voluntary isometric tension or more recently by Sale et al (1982). Dynamometer design must be such that the limb can be firmly fixed with the forces generated contained within the system. It must be possible to set accurately joint position to ensure muscle length is fixed. The transducer used must have a high resonant frequency and must be rigidly attached to the subject to avoid distortion of the signal.

Dynamometer

The dynamometer used in the experiments reported in this thesis was comprised of a rigid L-shaped steel frame and adjustable bench (Fig. 2.1). Subjects were seated on the bench with thigh horizontal and lower leg within the upright portion of the frame at an ankle angle of 85° . Differing upper leg lengths were accommodated by adjusting the position of the bench relative to the frame and the backrest relative to the bench. Lower leg length variation was accommodated by altering the number of boards placed under the foot. A shaped plate

LEG DYNAMOMETER

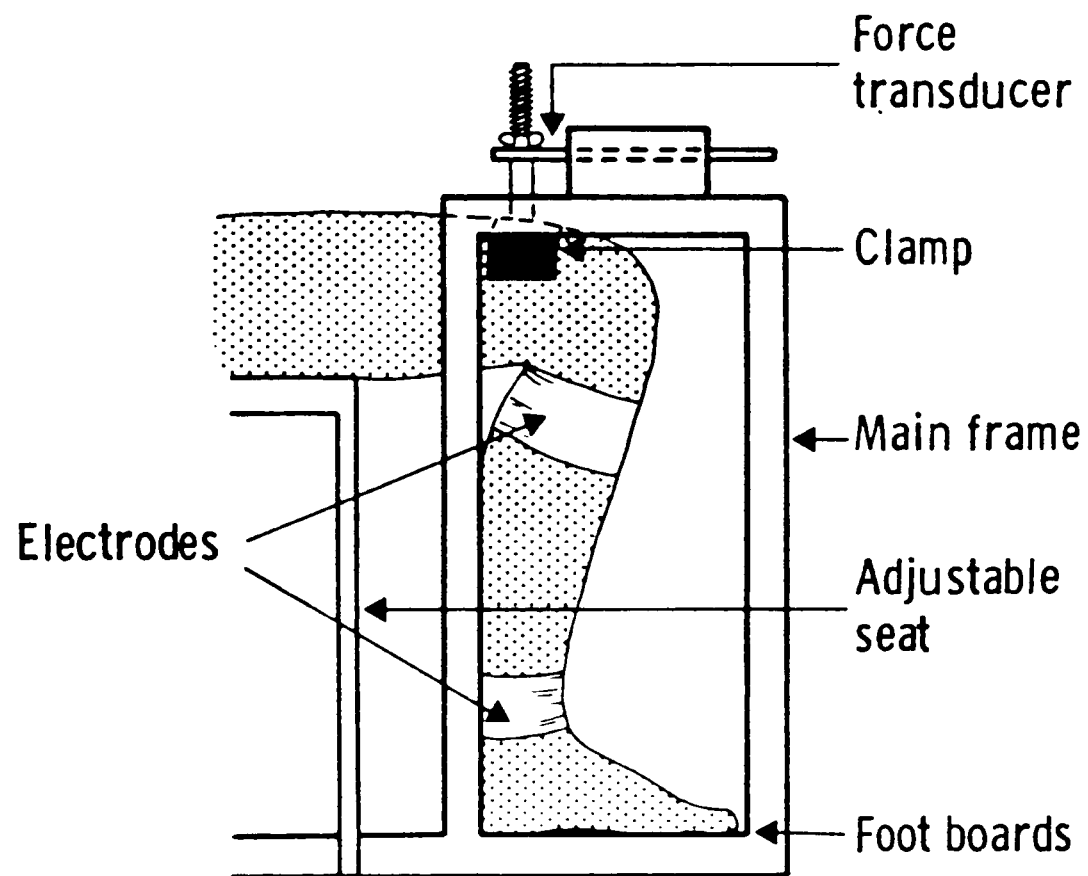


Figure 2.1.

Diagram of the leg dynamometer. The adjustable seat could be moved relative to the main frame before being clamped to it.

tightened down on to the thigh above the knee joint ensured near isometric conditions and transmitted upward force to a transducer mounted on the frame. The transducer (resonant frequency 500Hz) was a thick steel bar 3.2 cm x 1.2 cm in section, on to the upper and lower surfaces of which were bonded initially 1 and later 2 strain gauges (R.S. Components, foil type polyesterencapsulated). The strain gauges formed a wheatstone bridge circuit which was balanced (Fylde mini balance) the output from the circuit was amplified (Fylde mini amp) and displayed on a U.V. recorder and storage oscilloscope. Calibration of the dynamometer was by 10:1 lever system mounted above the transducer and output was linear up to an applied force of 2,500 N.

Electrical stimulation

The electrodes used were two wet ^(tapwater) 10 x 8 cm foil and tissue pads. Subjects reported least cutaneous sensation when the anode was placed over the heads of the gastrocnemius and the cathode over the belly of the soleus. Square wave pulses of 50 or 100 μ sec duration were delivered by a high voltage stimulator (Digitimer type 3072) controlled by a timer (Digitimer D 4030). In some experiments the actual voltage drop across the electrodes was measured using an oscilloscope. However, this was not done routinely as it destroyed the electrical isolation of the subject for the period of time the connection was made.

Standard Stimulation Protocol

Isometric twitches were evoked by single stimuli of increasing voltage at a rate of 1 every 30 sec continuing until no further increase in twitch force occurred. In this way stimulus voltage was increased from threshold levels, when few muscle fibres are recruited, to maximal levels, when all muscle fibres are recruited, and finally to supramaximal levels to ensure full recruitment. Twitch tension (Pt) time to peak tension (TPT) and half relaxation time ($1/2$ RT) were measured from U.V. records of the maximal responses.

Tetanic contractions were evoked by stimuli at frequencies of 10, 20 and 50 Hz for 2 sec at each frequency in a 6 sec stimulus train. Voltage was progressively increased after each train until force reached maximum or the subject requested that it should not be increased. A 1 min rest was given between trains. The criterion adopted as indicating that the tetanic response was maximal was that forces produced by three consecutive increases in voltage should agree within $\pm 5\%$. Some subjects especially in the first experiments carried out using this technique were unable to tolerate supramaximal stimulation at 50 Hz. In these instances the 50 Hz stimulation was removed from the train at submaximal voltages allowing 10 and 20 Hz stimulation to be continued to supramaximal voltages.

After a 2-3 min rest period 3 maximal voluntary contractions were performed separated by 30 sec. During all efforts the subject was vocally encouraged and able to see his response on the oscilloscope. The best effort was taken for analysis.

Finally, after a further 2-3 min rest period an electrically evoked fatigue test was performed. This comprised repeated tetanic contractions produced by supramaximal stimulation at 20 Hz for 300 msec once per sec for 2 min.

Electromyogram recording

In some experiments synchronous muscle action potentials evoked by supramaximal stimulation were recorded. The method used was similar to that of Hultman and Sjöholm¹¹ (1983) and is described in detail in Chapter IV Section 1.

All subjects gave informed consent and all procedures were passed by the ethical committee of the Queens Medical Centre, Nottingham.

2 - The Contractile Properties of the Human Triceps Surae: the effects of Passive and Active Change in Muscle Temperature

Introduction

Temperature has been shown to profoundly affect the time course and force of contraction as well as fatiguability in animal and human muscle. The magnitude and direction of the change induced depending on the exact temperature and type of muscle fibre involved (Bennett, 1984 and 1985).

Cullingham et al (1960) found the maximal tetanic tension of cat tibialis anterior occurred at temperatures of 38° to 40°C, on reducing muscle temperature to 28°C tension declined by only 5% but fell by 30-35% when temperature was further reduced to 20°C. Truong et al (1964) also found maximal tetanic tensions at 40°C in rat triceps surae but found a direct linear decline with temperature down to 15°C. Twitch tension showed an inverse linear relationship with temperature over the range 20°-40°C while, TPT and 1/2 RT showed a non linear inverse relationship with temperature. In the fast flexor hallucis longus of the cat Buller et al (1965) also found an increase in Pt, of 32% when muscle temperature declined from 37-38°C to 27°-28°C and an increase in TPT of 50-60%. However, in the slow soleus muscle Pt declined by 22% with the same temperature fall, though TPT increased by a similar amount to that seen in the faster muscle. Maximal tetanic tension was never reduced by more than 10% in either muscle over this 10°C temperature range. Close and Hoh (1968) showed that in the rat, the fast twitch extensor digitorum longus, while increasing twitch tension when cooled from 35 to 20°C continuously reduced tetanic tension over this

same temperature range. In contrast the slow twitch soleus maintained a constant twitch tension over the muscle temperature range 35 to 20°C and maintained a constant tetanic tension until the muscle temperature fell below 25°C.

In general it seems that faster muscles increase their twitch time course and tension as temperature declines but slow muscle while also increasing twitch time course under the same conditions can maintain or decrease twitch tension. Tetanic tension is affected by temperature in a species and fibre specific manner but if cooled below 25°C will always decrease (Bennett, 1984 and 1985).

Studies of the effects of temperature on human muscle have concentrated in the main on maximal voluntary force and fatiguability. Clarke et al (1958) found maximal hand grip strength was unaffected by temperature over the range 39-27°C but declined with further muscle temperature fall. Maximum endurance time holding a force of > 1/3 MVC was found at a muscle temperature of 27°C declining at higher and lower temperatures. Edwards et al (1971) also found significantly shorter endurance times at a muscle temperature of 38.6°C than at 31.6°C when holding 2/3 MVC in quadriceps.

Data concerning the effects of temperature on the triceps surae in man are confined to time course changes in the ankle jerk or twitch responses from small groups of fibres. Lambert et al (1951) found that the ankle jerk caused by a reflex synchronous action potential produced by Achilles tendon tap was prolonged as temperature dropped. The force of contraction would obviously depend on the number of muscle fibres recruited

and therefore was not controlled sufficiently in these experiments to give meaningful results. Petajan and Watts (1962) measured only the movement of the foot after reflex excitation, and found it slower after cooling. In the study of Buchthal and Schmalbruch (1970) twitch contractions of bundles of fibres were evoked by intramuscular stimuli in the end plate zone. Force was measured using a strain gauge attached to a needle stuck into the tendon. Again, absolute force measurement was not possible with this technique and only the time course of the twitch was reported. In three bundles of fibres studied in gastrocnemius TPT increased as temperature was decreased from 37°C to 25°C.

The information available is inadequate to predict the exact effects of temperature on the force and time course of isometric contraction of the human triceps surae, with various Q10 values reported depending on the temperature range studied (Petajan and Watts, 1962; Buchthal and Schmalbruch, 1970). As this could confound the interpretation of the results of the experiments performed in this thesis, especially those involving dynamic exercise when muscle temperature is bound to increase (Saltin et al, 1968) a direct study of the effects of passive and active changes in muscle temperature was undertaken. A further objective of the study was the evaluation of the reproducibility of the force and time course of the responses evoked by supramaximal stimulation.

Methods

The subjects were five men aged 21.9 ± 1.6 yr weight $75.3 \pm$

5.4 kg and height 183.5 ± 5 cm. They were habituated to the involved procedures particularly supramaximal tetanic stimulation during two visits to the laboratory before definitive measurements were made. The apparatus and stimulation protocol used were as described in Chapter II section 1. Following a 30 min rest period in the laboratory, control measurements were made after which muscle temperature was measured at 5 depths in the lateral belly of the gastrocnemius using a 5 cm thermistor probe - *sterilized in alcohol.*

The needle was inserted to its full 5 cm depth, then withdrawn in 1 cm steps as indicated by gradations on the needle shaft. At each depth the voltage displayed on a digital voltmeter was recorded once a steady reading was obtained. The thermistor was calibrated by immersion in stirred water at a range of known temperatures from 0°C to 50°C and gave a linear response. Lower leg temperature was then adjusted by above the knee joint immersion in a water bath or by exercise. Muscle temperature was remeasured immediately following leg immersion/exercise and again at the end of the experimental phase.

To maintain the adjusted temperature the leg was returned to the water bath or further exercised for 10 min mid way through the experimental phase following the measurement of the twitch responses. The range of water temperatures used was limited by subject acceptability to between 0°C , using iced water and 46°C . Immersion time was fixed at 30 min for heating but two different periods for cooling were used 20-30 min and 30-45 min. The final temperature achieved after 30 min immersion at 46°C was reproduced actively by requiring the subjects to run at 7 mph 0% gradient on a treadmill for 15 min.

Under all conditions the time course of contraction was described by the measurement of TPT and 1/2 RT from maximal twitch records. Force of contraction was measured from maximal evoked twitch, and tetanic responses at 10 and 20 Hz as well as maximal voluntary contractions. Fatiguability was assessed by the loss of force observed during the 2 min fatigue test. It was described by a fatigue index calculated as, the force evoked by the last stimulus train divided by the force evoked by the first train of the test.

Results

Mean temperatures at 5 depths below the skin under control and experimental conditions for the five subjects are shown in Fig. 2.2. illustrating the existence of a temperature gradient across the leg. Taking the mean of the three innermost readings at 5, 4 and 3 cm below the skin, in each subject, shows that the mean muscle temperature (T_m) of the lower leg at rest was $36.5 \pm 0.6^\circ\text{C}$. Immersion in water at 46°C increased T_m to $38.9 \pm 0.1^\circ\text{C}$ while exercise increased T_m to $39.1 \pm 0.4^\circ\text{C}$. There was no significant difference ($p > 0.1$) between the T_m achieved by active and passive heating. Cooling the leg by immersion in iced water for 20-30 min reduced T_m to $29.5 \pm 0.5^\circ\text{C}$ and a further reduction to $24.3 \pm 1.0^\circ\text{C}$ occurred after 30-45 min immersion.

Twitch responses

The mean time to peak tension (TPT) and half relaxation time (1/2 RT) for the five subjects under control conditions were $107 \pm$

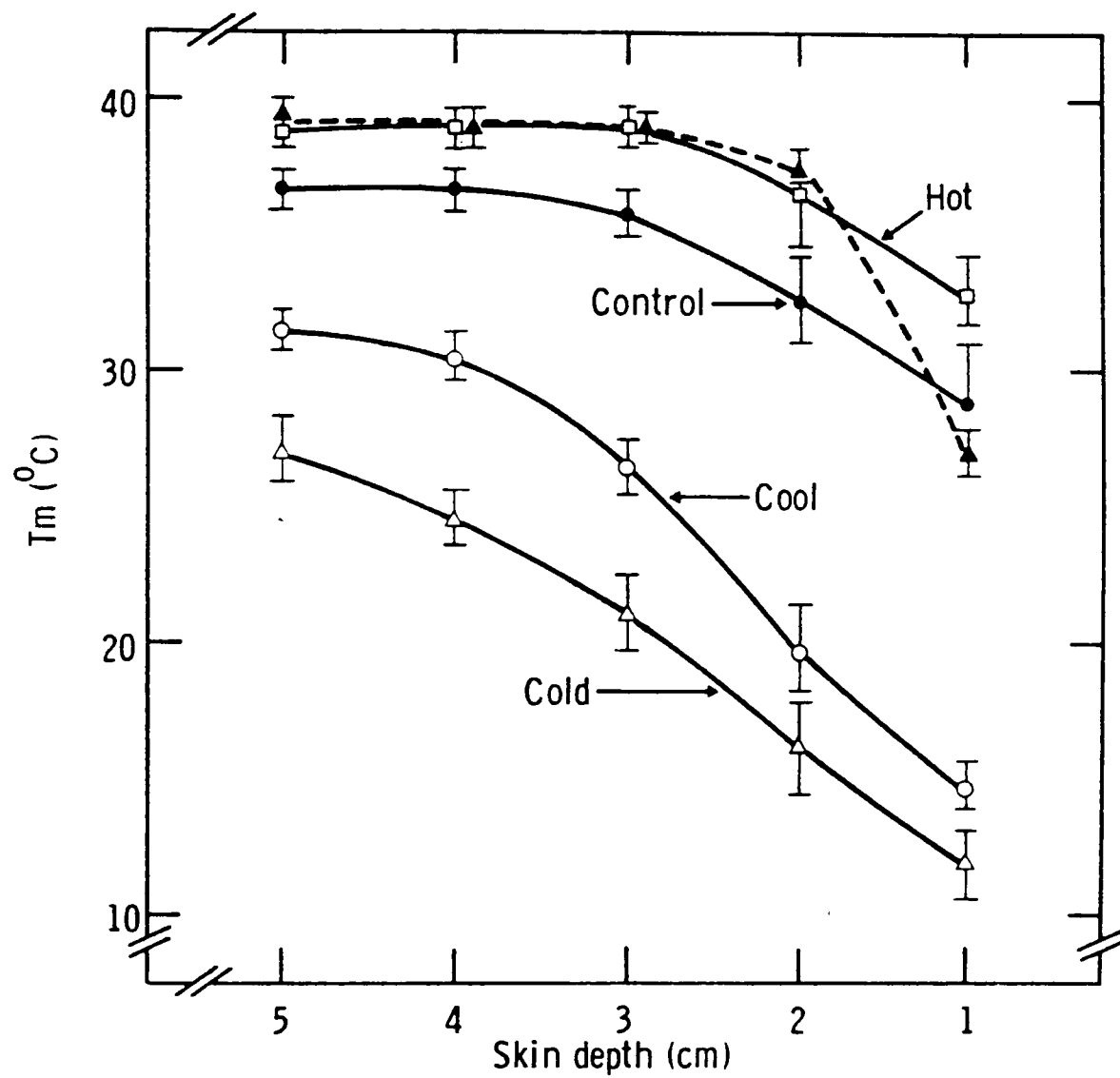


Figure 2.2.

Muscle temperature at 5 depths below the skin.

Symbols; control (\bullet); post exercise (\blacktriangle); heating (\square) and cooling (\circ, Δ) mean (\pm S.D.) data. (n=5)

13 msec and 76 ± 10 msec respectively. Mean intra-subject variability for TPT was $6.7 \pm 4.8\%$ and for $1/2$ RT was $10.6 \pm 4.2\%$. Twitch tension (Pt) showed a mean intra-subject variability of $8.3 \pm 3.4\%$ and a mean value of 102 ± 25 N.

The effects of heating and cooling on the twitch, stimulus-response curve are exemplified in Fig. 2.3. and the mean data for the maximal twitch responses are given in Table 2.1. Heating enhanced the twitch response at submaximal voltages, moving the stimulus response curve to the left, but did not significantly affect the maximal twitch tension while, TPT and $1/2$ RT were significantly reduced.

Both cooling conditions depressed submaximal and maximal Pt and prolonged TPT and $1/2$ RT. The differences in Pt, TPT and $1/2$ RT between cool and cold conditions were not significant. A strong inverse relationship was found between muscle temperature and TPT given by the equation:

$$\text{TPT(msec)} = -7.62.T_m(^{\circ}\text{C}) + 383.4, r = -0.98$$

and $1/2$ RT given by the equation:

$$1/2 \text{ RT(msec)} = -6.25.T_m(^{\circ}\text{C}) + 307.9, r = -0.96$$

From these equations values of TPT and $1/2$ RT were predicted at 29 and 39°C . These figures gave Q₁₀ values of 1.88 for TPT and 1.97 for $1/2$ RT over this temperature range.

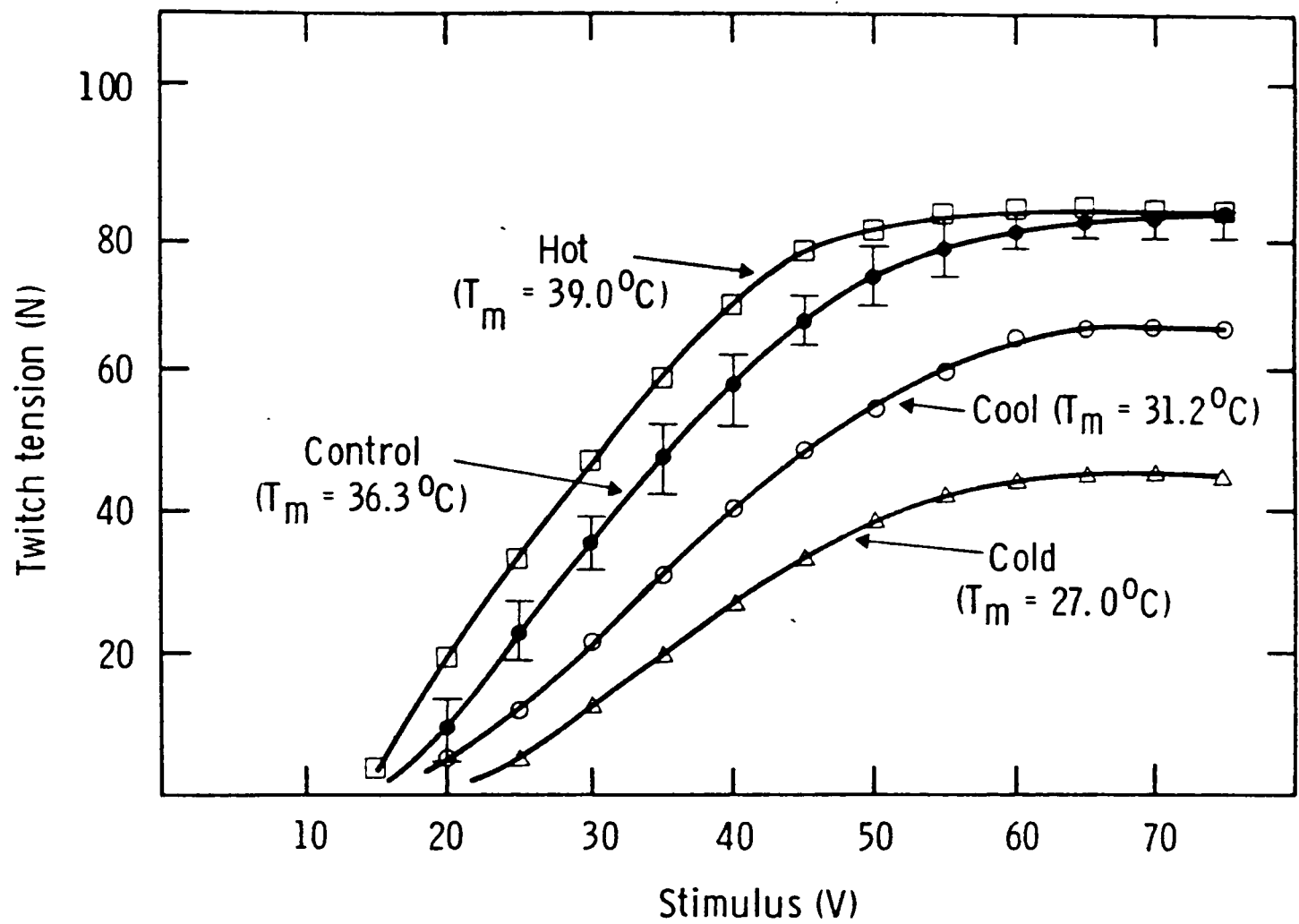


Figure 2.3.

Twitch Tension; the response to heating and cooling. The deep muscle temperatures are indicated in brackets.

Symbols: control (\bullet), hot (\square), cool (\circ) and cold (Δ).

Typical data one subject

TABLE 2.1.

Contractile properties of the triceps surae - effects of heating, cooling and exercise; Time to peak Tension (TPT); Time to half relaxation ($\frac{1}{2}$ RT). Maximal twitch tension (P_t), Maximal tetanic tension at 10 Hz (P_{O10}) and 20 Hz (P_{O20}) and maximal voluntary contraction (MVC). Mean \pm SD data for 5 young subjects.

Condition	TPT (msec)	$\frac{1}{2}$ RT (msec)	P_t (N)	P_{O10} (N)	P_{O20} (N)	MVC (N)	Condition	TPT (msec)	$\frac{1}{2}$ RT (msec)	P_t (N)	P_{O10} (N)	P_{O20} (N)	MVC (N)
Control	100 ± 19	78 ± 16	104 ± 35	752 ± 204	1156 ± 210	1852 ± 352	Control	110 ± 10	76 ± 6	101 ± 25	732 ± 158	1214 ± 202	1799 ± 302
Passive Heating	80*** ± 14	62** ± 13	103 ± 35	637*** ± 240	1154 ± 242	1892 ± 270	Exercise	92*** ± 13	64* ± 5	96 ± 24	602* ± 181	1063** ± 228	1765 ± 275
Control	108 ± 13	75 ± 13	101 ± 34	759 ± 189	1270 ± 225	1793 ± 214	Control	108 ± 16	74 ± 13	102 ± 29	742 ± 149	1158 ± 148	1816 ± 222
Passive Cooling	174*** ± 5	140*** ± 12	60** ± 16	801 ± 128	1207 ± 209	1562 ± 222	Passive Cold	188*** ± 16	146*** ± 29	49*** ± 14	732 ± 87	1072* ± 96	1493*** ± 254

Significance Control - exercise, Control - passive heating and cooling.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Tetanic Responses

Under control conditions the mean values for 10 Hz force, P_{o10} and 20 Hz force, P_{o20} were $748 \pm 163\text{N}$ and $1196 \pm 175\text{N}$ respectively. Mean intra subject variability was $6.7 \pm 2.9\%$ for P_{o10} and $7.9 \pm 1.9\%$ for P_{o20} . Again the effects of heating and cooling on the maximal responses are summarised in Table 2.1.

Cooling reduced the apparent fusion frequency of the triceps surae and responses to supramaximal stimulation were maintained at control levels except at the lowest T_m where there was a small but significant ($p < 0.05$) fall in P_{o20} .

Heating enhanced the 10 and 20 Hz responses at submaximal voltages but did not change the maximal 20 Hz response. However, there was a significant ($p < 0.001$) fall in the maximal 10 Hz response.

Maximal Voluntary Contractions

The repeated measurements of the maximal voluntary contraction (MVC) on each control occasion gave a mean value of $1832 \pm 247\text{N}$ and a mean intra subject variability of $8.4 \pm 0.6\%$. Neither heating nor cooling to 29.5°C had any effect on the force generated in an MVC but at a temperature of 24.3°C MVC was significantly ($p < 0.001$) reduced by an average of 17.8%.

Fatigue Test

The mean fatigue index (FI) for all subjects calculated as the force of the 120th tetanus divided by that of the 1st tetanus was 0.63 ± 0.09 under control conditions. F.I. showed a mean intra subject variability ($\pm 7\%$) of the same order as that found for P_{020} . Heating had no effect on the pattern of force loss during the test (FI 0.64 ± 0.05 , $p > 0.1$) but cooling significantly reduced the initial force and the decline of tension during the 2 min test period giving Fatigue indices of 0.81 ± 0.05 and 0.79 ± 0.06 under cool and cold conditions respectively Fig. 2.4.

Effects of Exercise

The effects of moderate exercise designed to produce the same T_m as in the passive heating experiments are shown in Table 2.1. The changes in TPT, $1/2 RT$, and P_{010} were closely similar to those found for passive heating but a small significant ($p < 0.1$) fall in P_{020} was seen after running which was not found after passive heating. Stimulus response curves were all shifted to the left following exercise.

Discussion

These experiments show that reliable and reproducible measurements of the isometric contractile properties of the human triceps surae can be made using supramaximal electrical stimuli.

The maximal twitch responses recorded under control conditions had a mean TPT of 107 msec and $1/2 RT$ of 76 msec which are in agreement with previous measurements made using a variety

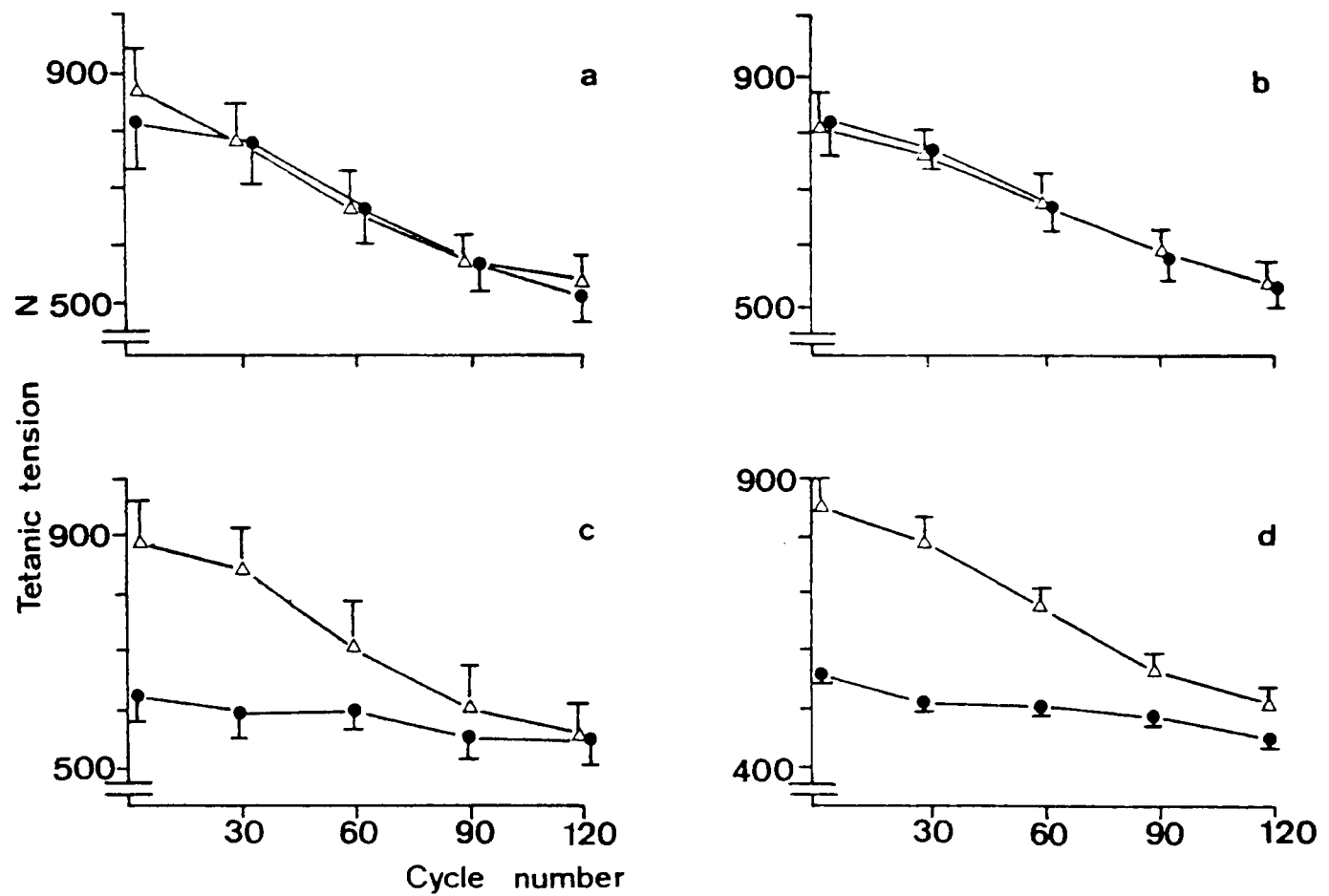


Figure 2.4.

Effects of intermittent stimulation for 300 msec at 20 Hz repeated once per second for 2 min on force generation under control conditions and (a) following warm up exercise (b) passive heating (c) cooling for 20-30 min (d) cooling for 30-45 min.

Mean data + S.E. control (Δ) and - S.E. experimental (\bullet) condition. (n=5)

of techniques. Indirect measurements made by Buller et al (1959), who recorded intramuscular pressure changes following local stimulation of a few fibres showed mean TPT to be 110 msec in gastrocnemius and 120 msec in soleus. McComas and Thomas (1968) using a bender element pressed onto the skin over the muscle belly, found a mean TPT of 118 msec and 1/2 RT of 84 msec in gastrocnemius during reflex twitch contractions. When twitch contractions were evoked by supramaximal stimulation of the popliteal nerve, Marsden and Meadows (1970) found a mean TPT of 104 ± 27 msec and 1/2 RT of 84 ± 14 msec, using a recording system which measured force generated at the ball of the foot and would therefore be expected to give similar results to the system used in the present work.

Bawa and Stein (1976) in human soleus, made similar observations to those of Rack and Westbury (1969) in cat soleus namely the dependence of twitch time course on muscle length as governed by ankle angle. Twitch time course, especially 1/2 RT was shown to become briefer as the muscle was shortened by moving the ankle from 77° in the human subjects. This effect has since been studied in maximal contractions of the whole triceps surae by Sale et al (1982) and it is believed to be due to a failure of activation at short lengths (Rack and Westbury, 1969). Choice of ankle angle is therefore a major factor in determining the TPT and 1/2 RT values reported, and underlines the need for careful standardisation. At angles above 90° it was possible for the foot to slip forward during stimulation and at angles less than 80° the heel became raised and was uncomfortable. In these studies an ankle angle of 85° was

chosen for subject safety.

Maximal tetanic stimulation at frequencies of 10 and 20 Hz was found to be acceptable in all five subjects after a period of habituation, usually 2-3 visits to the laboratory.

The fatigue test was a modification of that used by Burke et al (1973) to classify individual motor units in cat gastrocnemius. Here 40 Hz tetani lasting 330 msec were given 1/sec for up to 5 min to fatigue a unit. Force of the last tetanus divided by that of the first gave a fatigue index. Units which did not fatigue, F.I. near 1.0, and had relatively long contraction times were termed type S, faster units with F.I. near 1.0 were termed FR and units with low F.I. and faster contraction time were termed FF. Garnett et al (1979) used trains of stimuli at 10 or 20 pulses per second for 500 msec once per second or every 12 sec to assess the fatiguability of motor units in human gastrocnemius and also to attempt to classify motor units in a similar manner to Burke et al (1973). In this case the fatigue index was the ratio of the tension developed after 3,000 successive stimuli to the starting isometric tension. A design feature of both the Burke et al and Garnett et al fatigue tests was that no failure of the muscle action potential occurred during the test, avoiding the problems of force decline due to failure of excitation. The fatigue test used in this study of the contractile properties of the whole human triceps surae used 7 stimuli at a frequency of 20 Hz repeated once per second for 2 min a total of 840 stimuli which would not be expected to compromise muscle excitation (see Chapter IV section 2). Reproducible results were obtained with

this regime and a mean F.I. of 0.63 was found which taken together with an average TPT of 107 msec and the available histochemical data (Gollnick et al, 1974c; Edgerton et al, 1975a; Johnson et al, 1973) would indicate that in these young subjects the triceps surae behaves as expected of a mixed muscle with a predominance of slow fibres.

Effects of Temperature

Twitch

Bennett (1984) states that, "the maximal isometric twitch, (Pt), is a result of two competing sets of rate processes. One set, including activation and stretching of series elastic components, results in tension development. Another set involves termination of the active state and relaxation of muscle tension. The thermal dependence of Pt reflects a compromise of the thermal dependence of these numerous component rate processes. It is not particularly surprising therefore that the influence of temperature on Pt is so complex and variable". In animal experiments it has been consistently shown that over the range 40°C to 20°C, Pt increases as temperature declines in fast muscle of rat (Truong et al (1964); Close and Hoh (1968); Ranatunga (1982)) and cat (Buller et al (1965)) but in slow muscle Pt is unaffected by this temperature fall in rat, (Close and Hoh (1968); Ranatunga (1982)) or declines with temperature in cat, (Buller et al (1965)). The decline in Pt of the human triceps surae when cooled below 36.5°C in the present experiments would then be consistent with a predominantly slow

fibre population.

A uniform finding in all the animal studies above was an increase in the twitch time course of both fast and slow muscles as temperature was reduced. This was also found by Buchthal and Schmalbruch (1970) in twitches evoked from fast and slow bundles of fibres in human gastrocnemius and in the reflex contractions of the triceps surae by Lambert et al (1951) and Petajan and Watts (1962). Over the range of temperatures studied in the present experiments twitch time course of the whole triceps surae also changed in the same manner. The calculation of Q10 strictly applies only to rate processes (Bennett, 1984) those of the twitch are TPT and 1/2 RT. Calculated over the range 29^o-39^oC and assuming a linear relationship between temperature and time course the Q10 values were 1.88 for TPT and 1.97 for 1/2 RT in these experiments. The TPT value found lies between the value of 2.0 from 22-32^oC and 1.5 above 32^oC reported by Buchthal and Schmalbruch for the slow fibre bundles they studied. More importantly both the TPT and 1/2 RT Q10's lie exactly in the expected range for all mammalian muscle, over this temperature range, as calculated and compiled by Bennett (1984). Following exercise designed to produce the same Tm as in the heating experiments, by running for 15 min at approximately 70% $\dot{V}O_2$ max (Saltin et al, 1968) the TPT, and 1/2 RT were reduced and Pt remained unchanged. These effects were not significantly different from those described for passive heating and as muscle temperatures were also not significantly different in the two sets of experiments the same mechanisms are likely to be responsible for the changes under both conditions.

Tetanus

Cooling the triceps surae to 29.5°C had no significant effect on the maximal low frequency tetanic forces P_{010} and P_{020} or on the MVC. When T_m was lowered further to 24.3°C there was a significant decrease in MVC and P_{020} but not in P_{010} . It would seem that at the lower temperature the deleterious changes in the contractile process shown by reduced MVC and P_{020} and expected from both animal and human experiments, (Close and Hoh 1968; Clarke et al 1958; Bennett, 1984) are offset by a stimulus frequency of 10 Hz which normally produces a partially fused response. Perhaps because of the slower activation and deactivation rates (Bennett, 1984) at this temperature indicated by the prolonged twitch, a better summation of response is achieved resulting in a relatively more fully activated contraction than is seen under control conditions.

The effect of heating the muscle above control T_m would be expected to produce the opposite effect. Since activation and deactivation rates would increase resulting in a briefer twitch, summation of responses to 10 Hz stimulation would be reduced as would the level of activation decreasing P_{010} . In this way the significant fall in P_{010} found after passive heating and following exercise may be explained. Exercise also produced a significant fall in P_{020} which was not seen after passive heating so this must be a direct effect of the exercise. In the untrained subjects used in these experiments 15 min followed by a further 10 min of running at 7 mph may induce some muscle fatigue and as a result the responses to low frequency tetani may be preferentially affected (Edwards et al, 1977b). In the

absence of maximal high frequency responses this cannot be stated with certainty, however, the finding of a normal MVC after exercise would support this view.

Fatigue Test

Heating and exercise were without effect on the F.I. but cooling reduced the absolute force developed at the start of the test and decreased the rate of force loss during the test resulting in an increased F.I. Presumably, at 29.5°C slowing of rate of activation meant that the short 20 Hz tetanus used in the fatigue test, did not allow sufficient time for activation to reach a level such that normal force was developed even though summation should have been improved and at this temperature a 2 second 20 Hz tetanus generated normal force. Reduction of the temperature further to 24.3°C caused Po_{20} to fall even after a 2 sec tetanus and MVC was also reduced, this effect combined with slower rate of rise of activation would cause the loss of force produced by a brief tetanus under these conditions.

In the studies of Clarke et al (1958) and Edwards (1972) holding time of a fixed level of MVC was prolonged when muscle temperature was reduced indicating a greater fatigue resistance, ascribed to metabolic factors. The present results may compliment these findings but as the force/time integral was produced by cooling exact comparison is not possible.

In conclusion it can be seen that reliable measurement of maximal evoked responses can be made using the apparatus and techniques described. The contractile machinery of the triceps

surae appears unaffected by temperature over the range 39.1 to 29.5°C and is only modestly affected by further cooling to 24.3°C. On the basis of this evidence the fluctuations in intramuscular temperature expected in this large postural muscle under normal environmental and activity conditions, are unlikely to affect the capacity to generate force. However the processes controlling activation and deactivation of the contractile machinery are clearly temperature dependent. Thus the absolute force generated by unfused tetani and single twitch responses as well as the time course of such responses must be related to the muscle temperature at which measurement occurs.

CHAPTER III

The effects of disuse resulting from
involuntary and voluntary immobilisation

1 - Involuntary Immobilisation Following Injury

Introduction

Complete limb immobilisation produces muscle atrophy and weakness. In animal studies the degree of wastage can be assessed directly by measuring changes in the weight and cross sectional area of excised muscles, while the weakness can be demonstrated by a reduced response when the muscle is electrically stimulated (Booth, 1982). In man loss of limb bulk can be demonstrated by anthropometry, computerised tomography and ultrasound measurements (Häggmark and Erikson, 1979; Ingemann-Hansen and Halkjaer-Kristensen, 1980; Young et al, 1980). The results from the latter techniques reflect the decreases in fibre area seen in biopsy samples from atrophic muscle (Sargeant et al, 1977; Young et al, 1982). However, the measurement of muscle strength following immobilisation by the use of maximal voluntary contractions is especially difficult. In most studies the limb has been immobilised because of injury to a joint and/or bone, consequently on remobilisation there may be great reluctance to make a maximal effort for fear of re-injury. Until this fear has been overcome meaningful measurement of muscle strength cannot be made. The use of electrically evoked contractions would provide a method of assessing muscle function without reliance on subject motivation.

Alteration of relative fibre area may be responsible for the changes in whole muscle contractile characteristics seen in animal studies after immobilisation (Booth and Kelso, 1973). While similar changes in relative fibre area have been seen in human muscle (Häggmark and Eriksson, 1979; Edström, 1970;

Haggmark et al, 1981) there have been no measurements of evoked contractile characteristics following immobilisation in man.

The aims of this investigation were (1) to attempt to measure the electrically evoked twitch and tetanic characteristics of the human triceps surae after immobilisation following injury and (2) to relate the muscle forces produced by voluntary and evoked contraction to an anthropometric estimate of muscle cross sectional area.

Methods

The subjects were 11 servicemen aged 21.8 ± 3 years. All had suffered unilateral fracture of tibia and/or fibula and were recruited while at the Joint Services Medical Rehabilitation Unit R.A.F. Chessington, between January and July, 1982. They first visited the laboratory 36 ± 21 (range 10-72) days after becoming weight bearing on the injured leg and had been immobilised for 135 ± 68 (range 46-278) days. The subjects were habituated to the electrical stimulation techniques after four separate visits to the laboratory, during an initial 2 week period. Measurements of the contractile properties of the triceps surae of both legs were then made on the 11 subjects. During the following months some subjects were measured longitudinally (i.e. throughout their rehabilitation period) until discharged from the Unit. The apparatus and stimulation technique were as described in Chapter II section 1.

Anthropometric measurements were made with the subject standing before or at least 45 min after the stimulated contractions. Leg circumference was first ascertained at the widest point of the calf with a metal tape measure; the height

of this circumference from the ground was noted and all subsequent circumferences were measured at this position. Skinfold thickness at medial and lateral sites on the circumference were measured with standard calipers, and corrected after the method of Jones and Pearson (1969) before use in the calculation of muscle (plus bone) cross sectional area (CSA).

Results

All subjects tolerated maximal tetanic stimulation of the uninjured limb after two or three visits to the laboratory, but they were more protective of their injured leg and required at least a further two visits to habituate this limb to the involved procedure. In three subjects, however, maximal tetani could not be achieved, although maximal twitch responses were perfectly acceptable.

Table 3.1, shows the mean \pm 1 SD values obtained from injured and uninjured limbs on the first occasion the patients were able to tolerate maximal tetanic stimulation of their injured limb, or in the three cases where this was not possible when maximal tetani were first evoked from the uninjured limb. The half-relaxation time ($1/2$ RT) of the triceps surae was significantly increased, but the TPT was significantly reduced in the injured limb, and Pt was decreased by an average of 25%.

TABLE 3.1.

Comparison of values obtained from uninjured and injured limbs on the first occasion maximal tetanic stimulation was tolerated.

	TPT (msec)	1/2RT (msec)	Pt (N)	P _o 10 (N)	P _o 20 (N)	MVC (N)	FI	CSA (cm ²)	Circum- ference (cm)
Uninjured	118±16	92±14	114±31	696±207	1032±197	1462±198	0.66±0.16	89.2±13.9	37.0±2.8
Injured	88±16	108±16	86±32	353±87 n=8	558±126 n=8	738±214	0.70±0.15 n=8	74.8±11.3	35.0±2.6
Signif- icance	**	**	*	***	***	***	N.S.	***	***

Electrically evoked Pt, twitch tension; TPT, time to peak tension; 1/2 RT, half-relaxation time; P_o10, tetanic tension at 10 Hz; P_o20, tetanic tension at 20 Hz; FI, an index of fatigue; MVC, maximal voluntary contraction; 'circumference' indicates maximal calf circumference; CSA, cross-sectional area of muscle plus bone estimated anthropometrically. Significance: *p< 0.05; **p< 0.01; ***p< 0.001. N.S., Not significant.

The mean tetanic tensions at 10 Hz (P_{010}) and 20 Hz (P_{020}) were significantly ($p < 0.001$) decreased by 51% and 46%. This resulted in twitch tetanus (at 20 Hz) ratios (Pt/P_{020}) of 0.11 and 0.15 in the uninjured and injured legs respectively. MVC was reduced by an average of 50% in the injured limb compared with uninjured, reflecting the reductions in P_{010} and P_{020} (Fig.3.1.) Injured limb circumference was significantly reduced by an average of 5% when compared with the uninjured limb, but CSA minus skinfold thickness (muscle plus bone) was reduced by 16% in the injured limb. There was no significant difference between the fatigue index of the injured and uninjured limb, nor was there any correlation found between length of immobilisation and the reduction in twitch, tetanic or maximum voluntary force of the injured limb. However, the reduction (Δ) in injured limb TPT was related to duration of immobilisation (t). The relationship is given by the equation:

$$\Delta \text{ TPT (\%)} = 12.7 + 0.1097t(\text{days}); (r = 0.54)(p < 0.05)$$

Fig. 3.2. shows the relationship between calf CSA and P_{020} for injured and uninjured limbs on the first occasion maximal tetanic stimulation was tolerated on the injured limb. It will be noted that whereas the uninjured limb P_{020} was associated with CSA ($r = 0.47$) and closely related to previously established values the points for the injured leg lie below and are unrelated to the expected relationship for normal men. MVC showed considerably more variation than P_{020} and a reduced association ($r = 0.28$) with CSA in the uninjured limb. The MVC and CSA of the injured leg were not significantly ($p > 0.1$)

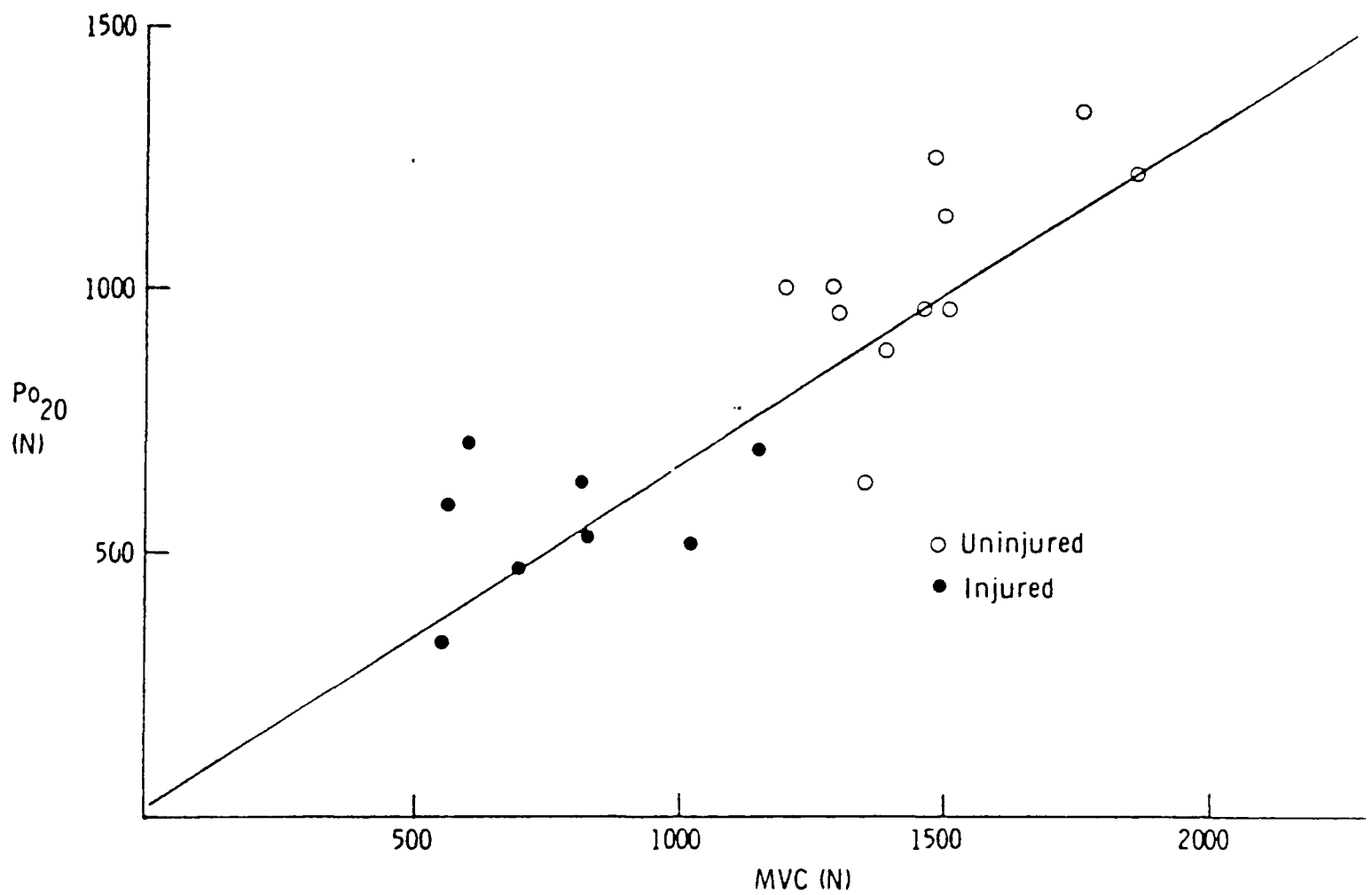


Figure 3.1.

Relationship between MVC and $P_{O_{20}}$. The solid line is the regression ($r=0.92$) for 30 subjects previously measured of a range of limb sizes. Note that $P_{O_{20}}$ and MVC are still related in the injured limbs in the present study.

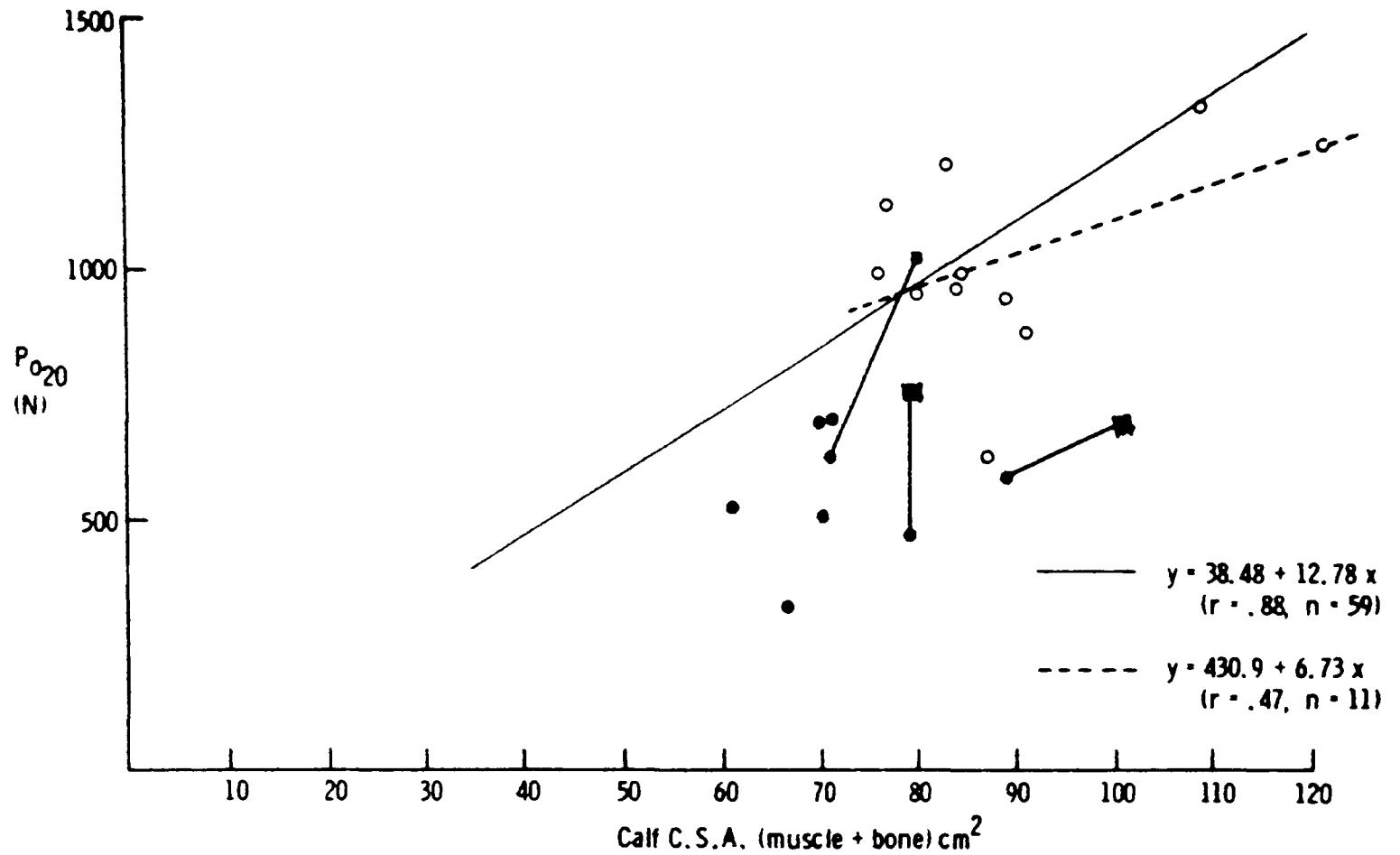


Figure 3.2.

Relationship between P_{020} and CSA in uninjured (o), injured (●) and recovering (■) limbs. The solid line indicates the regression line for 59 subjects previously measured of a range of limb sizes, the dotted line is the present uninjured limb regression line.

The lines linking two points show the change in Force and CSA during rehabilitation.

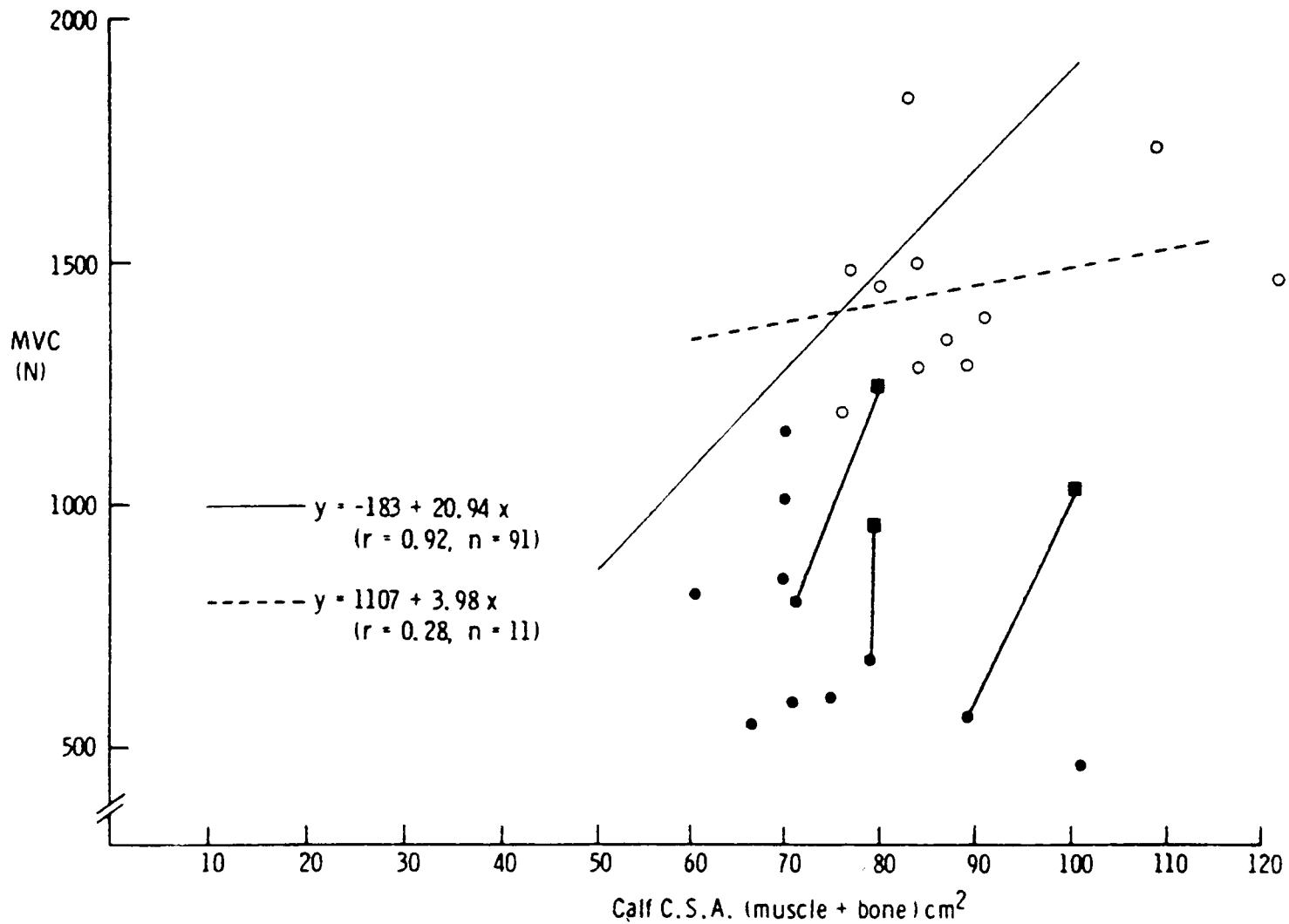


Figure 3.3.

Relationship between MVC and CSA. Symbols as Fig. 3.2. The solid line is the regression line for 91 subjects previously measured while the dotted line is for the uninjured limbs in the present study.

Again, the lines linking two data points show changes in Force and CSA during rehabilitation.

correlated (Fig. 3.3). In three subjects who were measured longitudinally (see the Methods section) both P_{020} and MVC of injured limb increased towards the values obtained in the uninjured leg. These increases were disproportionate to the change in CSA; indeed in one of the three subjects CSA remained constant through the period of rehabilitation (Figs. 3.2 and 3.3) The measurements of P_{010} showed the same pattern of change as P_{020} in both legs, and a similar relationship to CSA after injury.

Discussion

One of the major problems in assessing muscle strength after limb fracture and its recovery is overcoming the patient's fear of re-fracture. In the present study the patients had been weight bearing on the injured limb between 10 and 72 days before the stimulation techniques were applied, but they were still wary of any use of their injured limb, even though undergoing a well supervised rehabilitation programme. For this reason the habituation of the subjects to the evoked contractions took longer than the two or three visits required under normal circumstances, and, therefore, the initial values for the contractile properties in Table 3.1 were measured between 17 and 86 days after the patients' limbs were weight bearing. Nevertheless, a relationship between reduction in TPT and duration of immobilisation persisted, though no relationship between length of immobilisation and any of the other measured variables was found (cf. Sargeant et al, 1977). The reduced TPT of the injured limb relative to the uninjured limb may indicate

a general shift in the whole fibre population of the triceps surae towards a faster contractile speed, but if this were so then one would expect a reduction rather than an increase in $1/2$ RT (Table 3.1). A further possible cause of a decrease in TPT is decreased compliance of the system after immobilisation, which might also explain the increased twitch tetanus ratio observed, though $1/2$ RT would again be expected to decrease. However, a crude measure of muscle compliance made by moving the foot from full plantar flexion to full dorsiflexion showed that similar forces were required to move both feet in the subjects measured. The measurements were made with knee at right angles and lower leg horizontal, a spring balance attached to a strap was looped round the ball of the foot and used to pull the foot upright. A more likely explanation of the TPT change in the injured limb is a relatively greater atrophy of slower motor units, which make up the majority of the triceps surae (Johnson et al, 1973), as demonstrated in human quadriceps (Häggmark et al, 1981; Edström[#], 1970) and in rat gastrocnemius and soleus (Booth and Kelso, 1973; Booth et al, 1980) A loss of force attributable to slow motor units will reduce Pt and thus increase the relative contribution of faster firing units to force production and result in a decreased TPT (Fig. 3.4). Since slow units will still produce some force the relaxation phase of the twitch, though at a lower force, will follow the same time course as normal, consequently $1/2$ RT will be maintained or increased (Biscoe and Taylor, 1967) and total contraction time will be unchanged. Fig. 3.4 shows actual twitch records from the injured and uninjured legs of one individual, illustrating

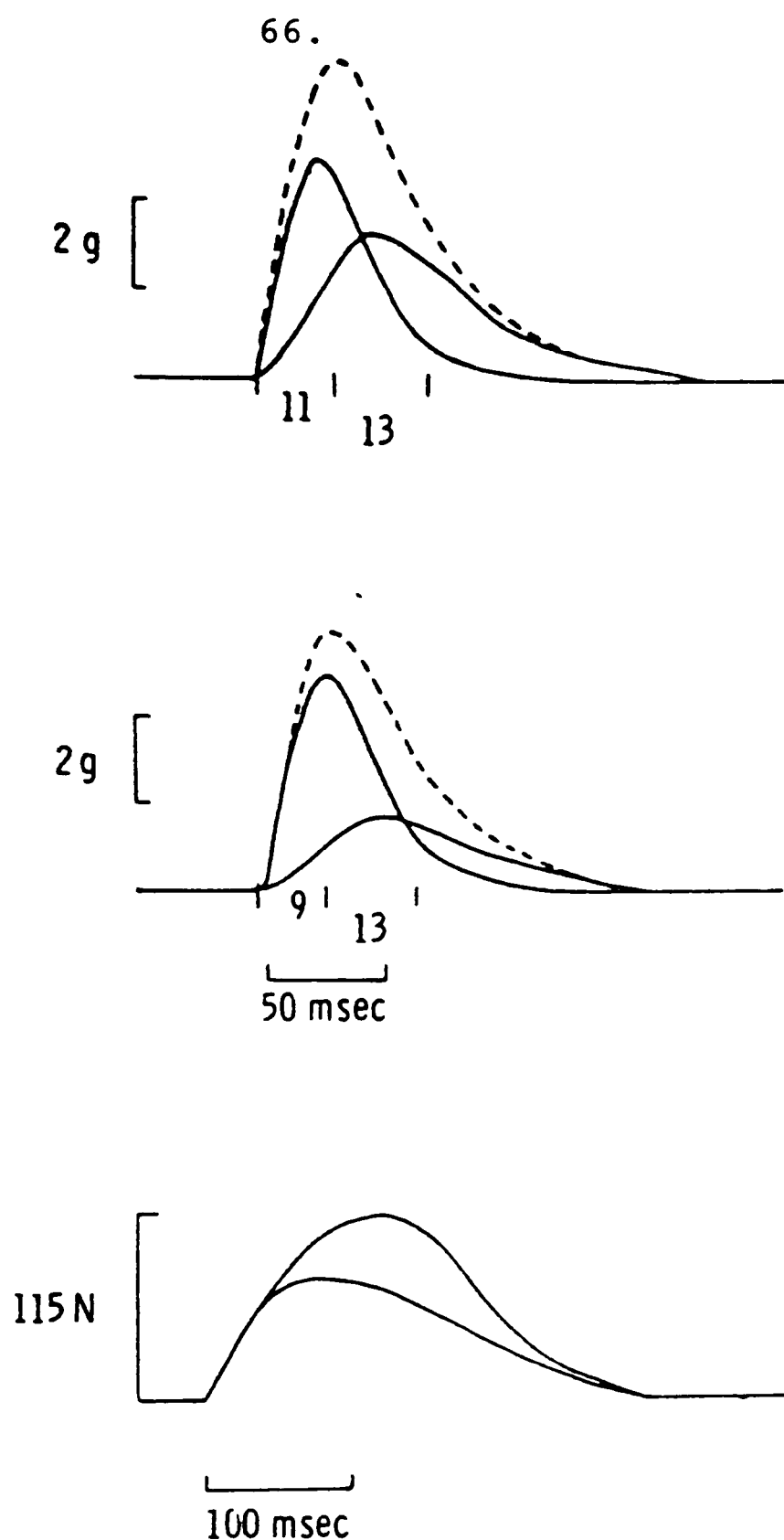


Figure 3.4.

Upper frame: Effects of a combination of a fast and slow twitch response, after Biscoe and Taylor. Dotted line shows resultant twitch, TPT 11 msec, $\frac{1}{2}$ RT 13 msec.

Middle frame: Effect of reducing the slow twitch by 50%. Note TPT is reduced to 9 msec, but $\frac{1}{2}$ RT is maintained at 13 msec, as is total contraction time.

Bottom frame: Actual twitch records from the uninjured (larger twitch) and injured (smaller twitch) limbs of the same individual.

reduced TPT, increased $1/2$ RT and maintained total contraction time in the injured limb. The reduced TPT in injured limbs may account for the slightly greater mean reduction in P_{O10} , of 51%, compared with 46% in P_{O20} , since fusion frequency would be expected to rise. That muscle forces, P_t , P_{O10} , P_{O20} and MVC are reduced by immobilisation is incontrovertible (Table 3.1); however, the relationships of the measured forces to estimates of muscle dimensions are not so clear. On theoretical grounds muscle force and physiological CSA must be related, and Fig. 3.2 shows that P_{O20} and the best estimate of CSA that can be made anthropometrically, calf muscle (plus bone) CSA are closely associated ($r = 0.88$), Uninjured limb P_{O20} and CSA also correlate, $r = 0.47$ (dotted line), with points distributed around the 'normal' line. However, injured limb P_{O20} and CSA are not associated, and the scatter of points is below the normal line. The distribution of points for the injured limb may be due, in part, to the anthropometric method for estimating CSA. Studies using ultrasonography (Young et al, 1980) and computerised tomography (Ingemann-Hansen and Halkjaer-Kristensen, 1980) have shown that gross measurements of muscle bulk can seriously underestimate the degree of atrophy within specific muscles of the leg. If this is the case in the present study then the simplest explanation for the reduction of P_{O20} by an average of 2.88 times as much as limb CSA in the injured limb, lies in the overestimation of the CSA of the force-producing muscles.

However, Haggmark and Eriksson (1979) found that after 6 weeks of immobilisation computerised tomography revealed a

similar percentage decrease in the calf muscles CSA as in the total lower leg muscle (plus bone) CSA. These findings imply that the changes in injured limb CSA in the present study do reflect the changes in calf muscle CSA and that the reason for the injured limb values in Fig. 3.2 lying below the normal line is a decreased specific tension. This has been shown in rat soleus (Witzmann et al, 1982; Fischbach and Robbins, 1969) and in cat gastrocnemius and soleus after tenotomy (Nelson, 1969).

The relationship between the injured leg MVC and CSA (Fig.3.3) must therefore be doubtful for the same reasons as for P_{O20} and CSA. In fact no correlation was found between MVC and CSA. However, the measurement of MVC on the injured limb appears to be a reliable indicator of muscle strength, as the mean fall in injured leg MVC is similar to the mean fall in P_{O20} , 50% and 46% respectively of uninjured leg values, and as Fig. 3.1 shows, MVC and P_{O20} are still normally related after immobilisation though the absolute values are lower. It must be remembered, however, that the subjects had habituated to the involved procedures over several weeks, and had become confident of using their injured limb by this stage. At earlier stages of rehabilitation injured leg MVC may not be a proper indication of muscle force-generating capability, as fear of refracture may inhibit voluntary activation of the triceps surae.

In the subjects who were measured longitudinally it can be seen that both P_{O20} and MVC can approach normal values after 9-11 weeks of measurement (Figs. 3.2 and 3.3). In fact in one subject P_{O20} of the injured leg was within the normal range of values, and equal to that of the non-fractured limb. However,

the injured leg MVC still did not match that of the uninjured leg, even though the subject was well motivated and training hard to pass his basic fitness test. This may indicate that a neural 'learning' has to take place before the maximum voluntary force can be generated (McDonagh et al, 1983; Ikai and Fukunaga, 1970; Davies et al, 1985). CSA increases are not so marked in all cases; indeed substantial increases in $P_{0.20}$ and MVC are clearly possible without measurable increase in limb CSA further highlighting the unsuitability of this measurement in assessing rehabilitation after lower limb immobilisation.

2 - Voluntary Immobilisation

Introduction

The preceding study raised several points. By showing no relationship between reduction in force generating capacity of the triceps surae (weakness) and duration of immobilisation period, it complements the work of Sargeant et al (1977). They found no relationship between degree of atrophy of muscle fibres, in biopsy samples from quadriceps, and length of time immobilised. This could be taken as evidence that there are changes occurring early in the period of disuse which persist unaltered throughout immobilisation. However, the fact that measurements could not be made until some time after the subjects regained the use of the injured limb may have allowed some recovery to take place. Further, because the immobilisation was unexpected due to accidental injury no control measurements could be performed. Comparison was therefore only possible between injured and uninjured limbs and no measure of absolute change in either limb could be made.

By studying the effects of a period of voluntary immobilisation in normal healthy individuals proper control measurements could be made. Since there is no injury, muscle function could also be assessed during and immediately after the period of immobilisation. The problem is the willingness of the subject to undertake such a study. In order to make it subject acceptable a reasonably short period of immobilisation is required. The following section describes a unique study which set out to investigate the immediate effects of a 14 day period of immobilisation on the contractile properties of the triceps surae

Methods

The subjects were four healthy men aged 25 ± 7 years who volunteered to allow one leg to be immobilised for a period of 2 weeks. The procedures for immobilisation and its attendant risks were considered acceptable by the ethics committee of Nottingham University Medical School. Full leg non-weight-bearing plaster of Paris casts were applied to the non-dominant (left) leg of each subject. Subjects were instructed that any discomfort or circulatory occlusion should be immediately reported and that the cast could be removed at any time at their request. They were told to try to use the limb as little as possible, especially attempting foot plantar flexion, but were given crutches to allow some mobility. Casts were applied and removed in the Fracture Clinic of the Queens Medical Centre, Nottingham.

On five occasions prior to immobilisation the electrically evoked and voluntary contractile properties of triceps surae, and the calf circumference and skin fold thickness at medial and lateral sites, of both legs were measured as previously described. All of the subjects were able to tolerate supramaximal tetanic stimulation at frequencies up to 50 Hz.

After 1 week of immobilisation the casts were removed and the measurements were repeated; a fresh cast was then applied for the second week, after which time the cast was removed and the measurements once more repeated. The subjects were then allowed to return to normal activity, with recovery measurements made at 2, 4, 7, 10 and 15 days after mobilisation of the limb.

Results

The effects of 14 days immobilisation are summarised in Table 3.2. There was a significant change in the time course of the twitch after 1 week of immobilisation. TPT was increased from $126 \pm 10\text{ms}$ to $143 \pm 20\text{ ms}$ ($p < 0.05$) and $1/2\text{ RT}$ increased from $88 \pm 7\text{ ms}$ to $107 \pm 12\text{ ms}$ ($p < 0.01$). This effect was unaltered by a further week of immobilisation.

Mean twitch tension increased over the 2 week immobilisation period, but the change was not significant. Evoked tetanic responses $P_{0.10}$, $P_{0.20}$ and $P_{0.50}$ were not significantly altered by 1 or 2 weeks of immobilisation, but the mean values were progressively reduced during this period, being 18, 13 and 10% respectively lower than in the control period at the end of immobilisation, and they were associated with the decreases seen in (muscle plus bone) cross-sectional area.

Cross-sectional area CSA of muscle plus bone was reduced by 5% ($p < 0.01$) after 1 week and a further 3% ($p < 0.05$) after 2 weeks. The fatiguability of the muscle was unchanged by the immobilisation, however mean MVC was progressively reduced by 11% ($p < 0.05$) after 1 week and a further 13% ($p < 0.05$) after 2 weeks.

The effects of immobilisation on TPT were short lived; the TPT values were not significantly different from normal 2 days after the plaster was finally removed. The $1/2\text{ RT}$ was not significantly different from normal after 7 days (Fig. 3.5). MVC also returned to normal rapidly, with no significant difference observed between control values and those measured after 4 days of recovery (Fig. 3.6). The recovery period of CSA was longer,

TABLE 3.2.

Effects of voluntary immobilisation on the contractile properties of the Triceps Surae. Mean \pm 1 S.D. n = number of observations.

	TPT (msec)	1/2RT (msec)	Pt (msec)	P _o 10 (N)	P _o 20 (N)	P _o 50 (N)	MVC (N)	FI
Control (n=20)	126 \pm 10	88 \pm 7	142 \pm 28	687 \pm 109	939 \pm 143	1177 \pm 190	1367 \pm 190	0.62 \pm 0.11
After 1 week (n=4)	143*(2) \pm 20	107*(3) \pm 12	147 \pm 32	677 \pm 185	912 \pm 256	1129 \pm 322	1210*(2) \pm 283	0.71 \pm 0.25
After 2 weeks (n=4)	145*(3) \pm 11	104*(2) \pm 14	161 \pm 41	561 \pm 98	815 \pm 144	1067 \pm 193	1042**(4) \pm 213	0.70 \pm 0.20

*Significantly different from control.

**Significantly different from control and week 1.

() Number of subjects in whom a significant change from their own control value was observed.

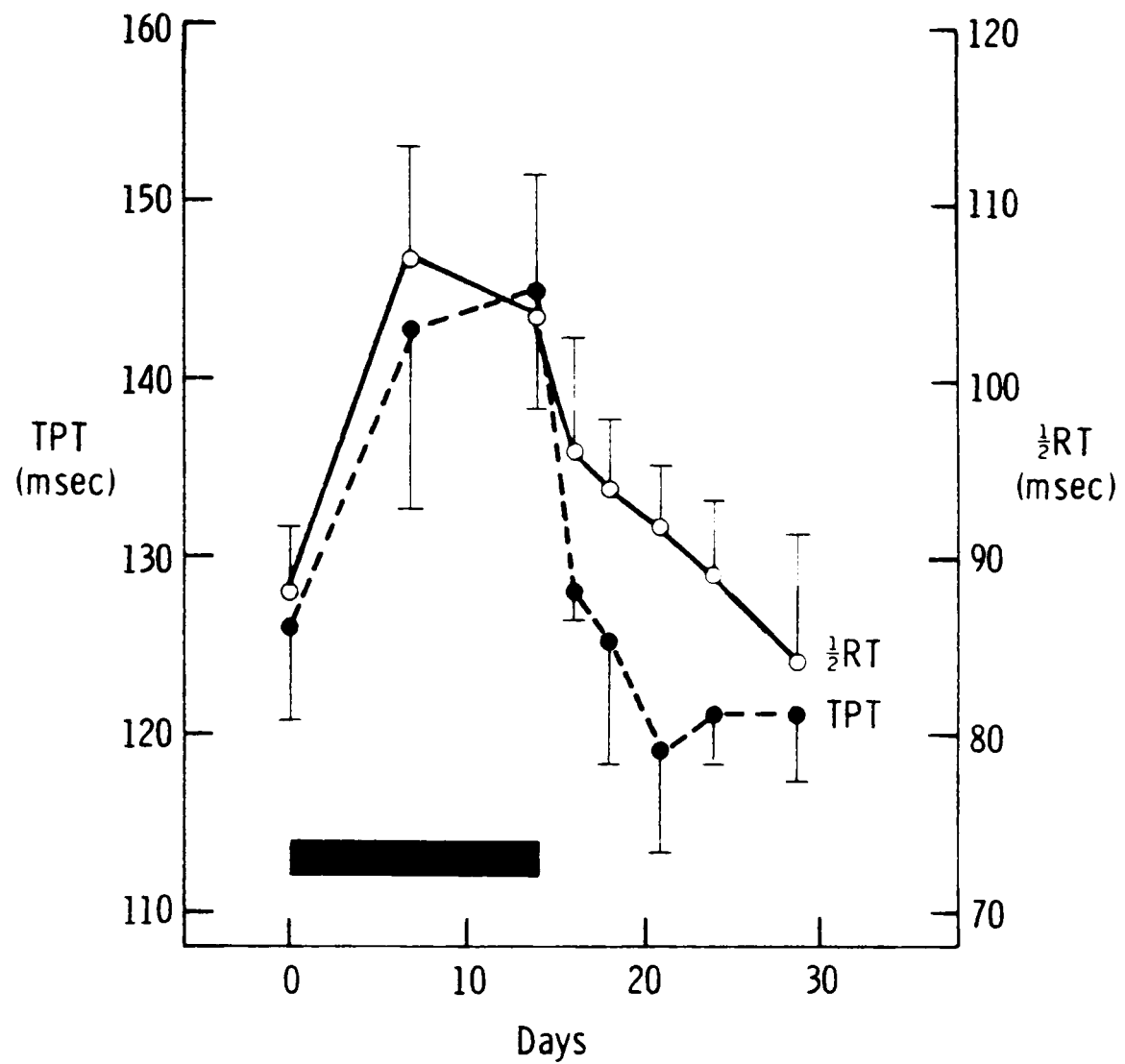


Figure 3.5.

The effect of 14 days voluntary immobilisation (filled bar) on the twitch TPT (●) mean - 1 S.E. and $\frac{1}{2}$ RT (○) mean + 1 S.E. of the triceps surae. $n = 4$. Recovery measurements are 2, 4, 7, 10 and 15 days after the end of immobilisation.

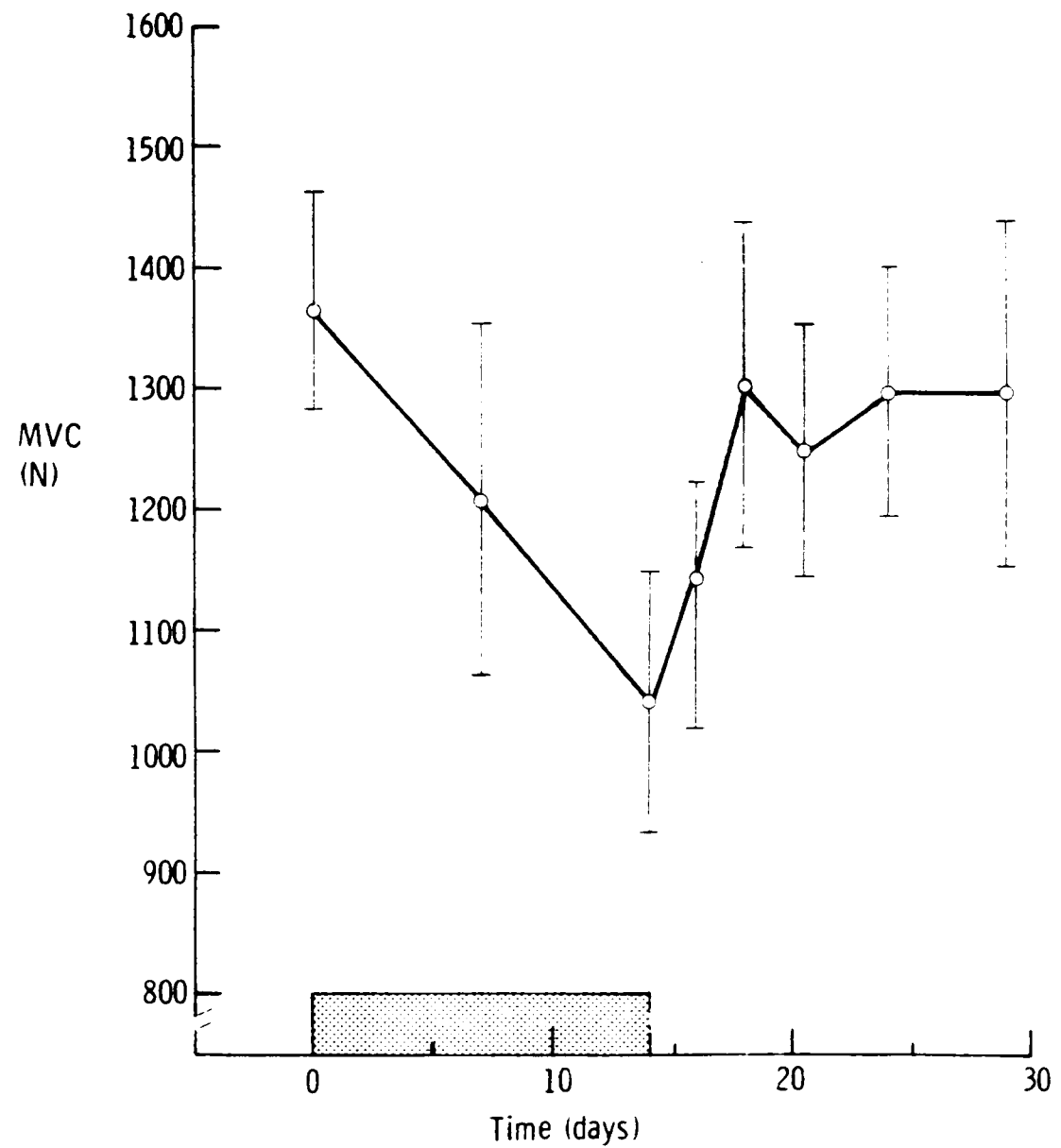


Figure 3.6.

Effect of 14 days voluntary immobilisation (hatched area) on the maximum voluntary contractile strength of the triceps surae. Recovery measurements as Fig. 3.5. $n = 4$, mean \pm S.E.

requiring 14 days of use to reach control levels (Fig. 3.7).

Throughout the immobilisation period and recovery period no significant changes were observed in any of the measured variables of the control leg (see Table 3.3).

Discussion

The results show that a slow contracting muscle group was rapidly affected by disuse. Significant increases in maximal isometric twitch TPT and 1/2 RT were produced after 1 week of immobilisation, and these effects were maintained during the second week. The increased TPT reported here is in contrast to the changes seen after long-term (over 6 weeks) immobilisation following injury when though total twitch contraction time was maintained the TPT decreased. It was suggested that the most likely explanation for these findings was a selective atrophy of type I fibres within the triceps surae sufficient to reduce P_t and TPT (Booth and Kelso, 1973). Bearing in mind that the long-term immobilisation study used patients who were fully weight bearing and had been rehabilitating their injured limb for several weeks before measurements were made, while in this study measurements were made within 1-2 h of cast removal and recovery occurred in a period of days, it is unlikely that the same mechanism (type I atrophy) is responsible for the changes observed. The explanation for the changes seen following short term immobilisation may be a reduction in the rate at which calcium is dissociated from the myofibrillar proteins (Briggs, et al, 1977). Dissociation would occur more slowly if the rate of calcium re-uptake by the sarcoplasmic reticulum was

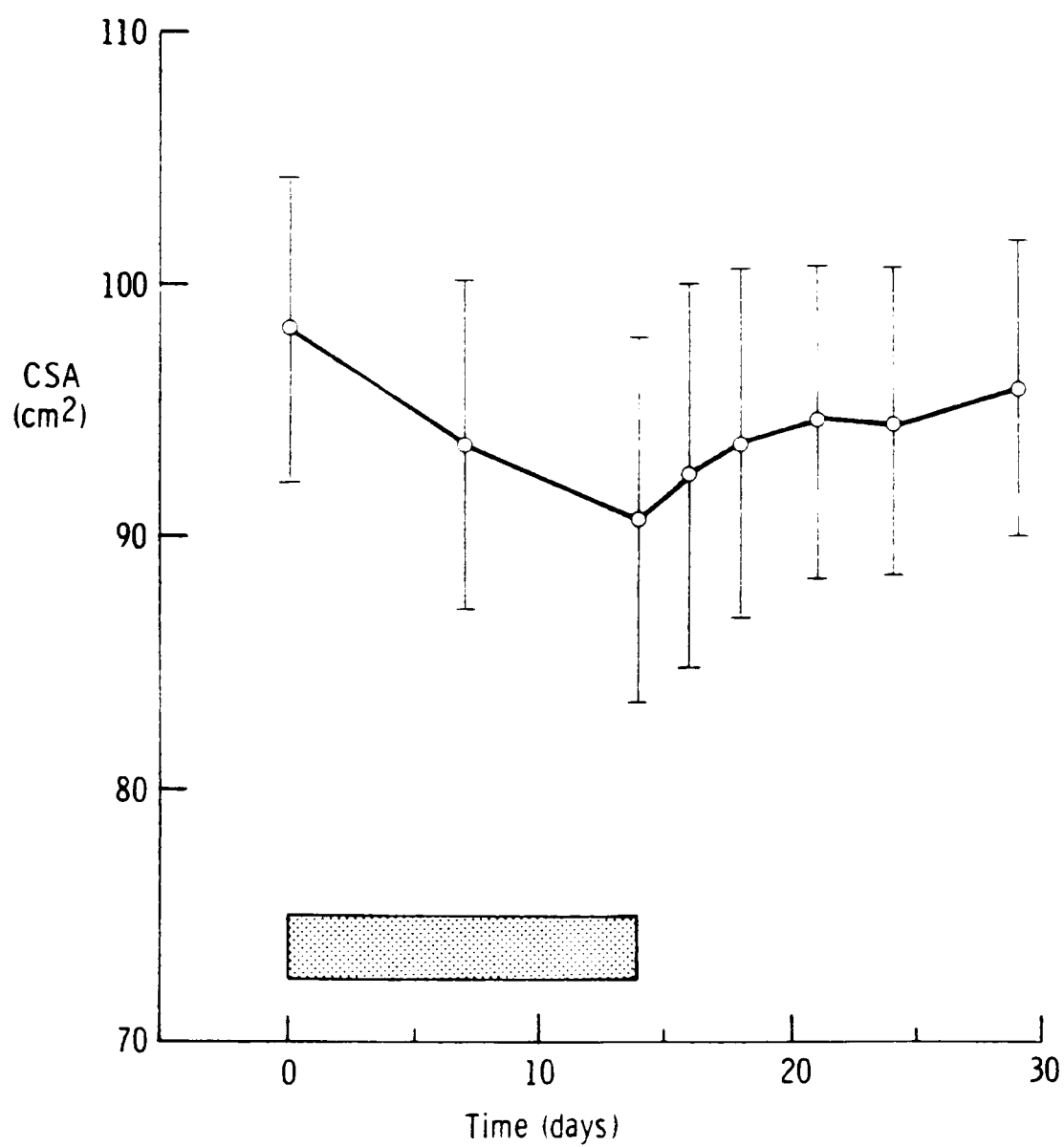


Figure 3.7.

Effect of 14 days voluntary immobilisation on the anthropometrically estimated calf cross-sectional area of muscle plus bone. Recovery measurements as Fig. 3.5. $n = 4$, mean \pm S.E.

TABLE 3.3.

Effects of 14 days voluntary immobilisation on the contractile properties of the triceps surae; control leg. Mean \pm 1 S.D.

	TPT (msec)	1/2RT (msec)	Pt (N)	P _o 10 (N)	P _o 20 (N)	P _o 50 (N)	MVC (N)	FI
Before	127 \pm 12	90 \pm 7	141 \pm 23	663 \pm 102	877 \pm 133	1098 \pm 192	1321 \pm 273	0.62 \pm 0.17
After	122 \pm 3	95 \pm 8	142 \pm 30	680 \pm 106	900 \pm 154	1091 \pm 228	1396 \pm 309	0.56 \pm 0.28

decreased. Such a decrease has been found following immobilisation of the rat soleus by Kim et al, (1982). A reduced rate of calcium dissociation from myofibrillar proteins might be expected to not only increase the time course of the twitch response but also allow more force to be generated, since cross bridges will continue to be formed while calcium is available. The tendency for P_t to increase in this study, though not significantly during the immobilisation, may, therefore, be explained. These effects on the sarcoplasmic reticulum would also be expected during long term immobilisation following injury, but would be difficult to observe. The effects on twitch force would be masked by atrophy and the rapid recovery of TPT and $1/2$ RT would necessitate measurements soon after the removal of the plaster, which is invariably unacceptable to the subject. The recovery of TPT and $1/2$ RT to normal within 2 and 7 days respectively (Fig. 3.5) is of interest since, assuming the twitch changes are due to sarcoplasmic reticulum alterations, they must reflect the trainability of the sarcoplasmic reticulum once tonic neural drive is restored and contrast with the retraining of other muscle enzyme systems (Houston et al, 1979).

The decrease in tetanic tensions at frequencies of 20 and 50 Hz failed to reach conventional levels of significance following the first and second week of immobilisation (Table 3.2), but changes in P_{020} and P_{050} (13 and 10% respectively) were of the same order of magnitude as the decline in CSA (8%) and the three variables were closely associated ($r = 0.84$). This implies that genuine atrophy of the muscle may have

occurred. However, some caution is necessary. In the previous study of long-term immobilisation following leg fracture the reduction in P_{010} and P_{020} was 51 and 46% respectively compared with a decrease in CSA of 16%; clearly a different relationship between tetanic force and CSA to the one found for this study. It is conceivable that in short-term immobilisation changes in CSA reflect alterations in fluid content rather than loss of contractile machinery, in which case the association found of force with CSA is one between two independent variables.

The much larger and progressive reduction in MVC when compared to the insignificant change in P_{050} after 1 and 2 weeks of immobilisation may indicate an inability of the central nervous system to activate the triceps surae normally. Whether this is due to a lack of motivation on the part of the subject, or an involuntary reduction in neural drive, is difficult to distinguish. The subjects certainly appeared well motivated and had no discomfort or knee stiffness before performing the tests, which could account for the low MVCs. Fischback and Robbins (1969) showed aggregate electromyogram in the rat soleus fell to 5-15% of control values on immobilisation, and if a similar effect occurred in the present study perhaps a period of neural 'retraining' was necessary during the 4 days after immobilisation before a normal MVC could be performed (McDonagh et al 1983; and Davies et al 1985). Though the absolute rates of protein synthesis and degradation under the present conditions are not known, the rate of protein turnover in normal (Halliday and McKeran, 1975; Rennie et al, 1982) man has been measured. Assuming degradation rate is normal in the recovery

period and synthesis rate increases as much as has been shown to be possible in catch-up growth in animals (M.J. Rennie personal communication), it is still inconceivable that net synthesis of myofibrillar protein could be high enough to account for the recovery of MVC in only 4 days. This further supports the non-significant effects on evoked tension development in indicating that immobilisation for 2 weeks had little effect on the contractile machinery of the triceps surae. The constancy of the measurements on the control leg (Table 3.3) show that there was no transfer of any effect from immobilised to control limb during or after the period of immobilisation.

CHAPTER IV

The Effects of Age on Muscle Function

1 - Contractile Characteristics

Introduction

Histochemical studies of elderly muscle in the 60-90 plus age group have shown that the frequency of neuropathic changes increases as anatomically more distal muscles of the leg are sampled (Jennekens, 1971; Tomonaga, 1977). The principal observation being that instead of the normal mozaic pattern of fibre type distribution, groups of fibres of the same type are seen. This is taken as evidence of a process of denervation and re-innervation by collateral sprouting from adjacent motor nerves (Gutmann and Hanzlíková, 1976). This view is complimented by the neurophysiological findings of Campbell et al (1973). They reported a decrease in the number of functioning motor units within the extensor digitorum brevis (E.D.B.) of men and women between 60 and 96 years. Each unit generated a larger than normal electrical response, and this was taken as evidence of expansion of the territory of the units to include a greater number of fibres. The technique used to estimate the number of motor units, was that of McComas et al (1971). Here each additional increment to the muscle M wave (the synchronous action potential), evoked by increasing electrical stimulation of the motor nerve is attributed to the recruitment of an additional motor unit. The mean amplitude of several increments divided into the maximum evoked M wave amplitude gives an estimate of the total number of motor units. It must be remembered that the size of an increment must depend not only on the number of fibres within a unit but also on the distance of those fibres from the recording site. A further assumption

which has to be made with this technique is that each increment is due to one unit only, and not several units with equal threshold. Using the same technique Sica et al (1976) found that in soleus there was again a decrease in the number of motor units with age. Reduction was gradual from 30 to 60 years but rapid between 60 and 90 years. However, in this study no change in the size of the average motor unit potential was found with increasing age, and they concluded that in contrast to the E.D.B. denervation without subsequent collateral reinnervation took place in soleus. A further implication of this finding is that there may be a loss of muscle fibres in soleus with increasing age. This has also been suggested by Grimby and Saltin (1983) from estimates of total muscle cross sectional area and mean fibre area, in 20 to 30 year olds and 80 year old men. Grimby and Saltin argue that since the loss of strength with age is of the same order of magnitude as the change in muscle mass, there is no reason to postulate qualitative changes in the remaining muscle or muscle fibres. This may well be true if maximal force alone is considered in relation to muscle cross sectional area (Young et al, 1984 and 1985). However other contractile characteristics of the elderly muscle clearly change. Campbell et al (1973) not only estimated motor unit numbers in E.D.B. but also recorded isometric twitch responses of the extensor hallucis brevis (E.H.B.), the most medial subdivision of the E.D.B. They found that the mean contraction and half relaxation times of the E.H.B. in the elderly 60-90 year old subjects were significantly longer than the values reported in a previous study for subjects 3-58 years old (Sica

and McComas, 1971). In the early study no relationship between age and twitch characteristics could be demonstrated. This was obvious evidence that most of the surviving motor neurones in the elderly E.H.B. innervated slow twitch fibres. What Campbell et al (1973) could not decide was whether the ageing process involved fast twitch units preferentially or whether the slowing of the twitch resulted from an alteration in the properties of the surviving innervated fibres due to excessive activity (Salmons and Vrbova, 1969).

A slowing of the activation and deactivation processes of contraction in some or all of the remaining innervated fibres would cause prolongation of the twitch and allow more time for force generation. This could contribute to the larger than expected twitch tension found by Campbell et al (1973) when they considered the number of motor units in the elderly E.H.B. However the explanation given of increased motor unit size is an equally valid conclusion on the basis of the experimental data. Only a study of individual motor unit properties could separate these two complimentary effects (Garnett et al, 1979).

Neuropathic changes such as fibre grouping and type II atrophy have been shown to occur in gastrocnemius after age 60 (Jennekens, 1971; Tomonaga, 1977). In the soleus, Sica et al (1976) showed the loss of functioning motor units accelerated after this age, but there was no evidence of reinnervation in this muscle.

If there are changes in fibre number and area in the triceps surae with age, then these changes would be expected to alter its contractile properties. The experiments in the next

chapter therefore set out to investigate the mechanical characteristics of the elderly muscle.

Methods

The subjects were 13 men, mean age 69.6 ± 1.3 years, height 1.68 ± 0.05 m and weight 73.6 ± 7.6 Kg. All were retired steelworkers recruited pre-retirement by the Department of Physiology and Pharmacology, Queens Medical Centre, Nottingham for a study of the effects of retirement on Health and Fitness. Before commencing the present study each subject was given a full medical examination and completed a questionnaire on their recent medical history. The presentation of signs or symptoms of cardiovascular disorder, particularly the absence of dorsalis pedis and posterior tibial pulse, or neuromuscular disorder were the criteria for exclusion of subjects from the study.

The dynamometer and recording system used to measure the forces produced by electrically evoked and voluntary contractions of the triceps surae were as described in Chapter II section 1. Measurements were made on all 13 subjects on three separate occasions and on a further two visits to the laboratory on 7 of the subjects.

Results

Table 4.1. shows the mean (\pm SD) data obtained on 7 subjects who made 5 visits to the laboratory. All subjects tolerated supramaximal single stimuli on the first and subsequent visits to the laboratory. Pt, TPT and $1/2$ RT showed no significant differences between the 1st and 2nd occasions of

TABLE 4.1.

Habituation of 7 elderly subjects to the experimental protocol over 5 visits

Visit	TPT (msec)	1/2RT (msec)	Pt (N)	P _o 10 (N)	P _o 20 (N)	MVC (N)
1	145±9	91±20	108±28	-	-	1,108±216
2	153±9	97±9	102±25	-	-	1,197±247*
3	152±17	101±12	108±29	613±149	826±175	1,274±233
4	150±19	90±11	110±26	583±112	782±122	1,256±265
5	158±13	110±13	106±26	576±178	761±188	1,237±173

TPT = Time-to-peak tension; 1/2 RT = half relaxation time; Pt = maximal twitch; P_o10 = tetanic tension at 10 Hz; P_o20 = tetanic tension at 20 Hz; MVC = maximal voluntary contraction. Significance 1st/2nd visit, * p < 0.05; 2nd-3rd/4th/5th visits, NS.

measurement or between the 2nd and 3rd, 4th and 5th occasions. Mean intra-subject variability was 5.4%, 9.1% and 12.5% for TPT, 1/2 RT and Pt respectively, over the five visits.

Maximal tetanic responses at 10 and 20 Hz (P_{010} and P_{020}) were obtained from 3 subjects on the first visit to the laboratory. On the second visit all the subjects tolerated supra-maximal 10 Hz stimulation and 6 of the 7 supra-maximal 20 Hz stimulation. After three visits all of the subjects tolerated supra-maximal stimulation at 10 and 20 Hz. Once habituated no significant difference was observed between the initial maximal response from an individual and subsequent maximal responses from the same individual, at either frequency. The mean intra-subject variability of P_{010} 10.3% and of P_{020} was 9.0%.

Comparison of the MVC measured on the 1st and 2nd visits to the laboratory showed a significant increase ($p < 0.05$). There was no significant difference between the values obtained on the 2nd and 3rd, 4th or 5th visits and mean intra subject variability over the five visits was 8.3%.

A total of 13 subjects were habituated to supra maximal twitch and tetanic stimulation, the mean data is given in Table 4.2. The values presented are the means \pm 1SD of the measurements made on the third visit to the laboratory. Five subjects tolerated supra-maximal 50 Hz stimulation on this their third occasion of measurement but were not members of the group tested on 5 visits to the laboratory, therefore this data does not appear in the earlier table. Included in Table 4.2 for comparative purposes are the mean values \pm 1SD for 9 subjects mean age 20.4 ± 1.4 years. These young men took part in the

TABLE 4.2.

Mean values of parameters measured on the third visit to laboratory, in 13 elderly men. Also included are the values for 9 young subjects used in the dynamic exercise studies.

	TPT	1/2RT	Pt	P _o 10	P _o 20	P _o 50	MVC	FI	Pt/P _o 50	Wt
	(msec)	(msec)	(N)	(N)	(N)	(N)	(N)			(Kg)
Old n=13	148 ± 16	97 ± 12	88 ± 32	519 ± 149	710 ± 175	838 ⁿ⁼⁵ ± 65	1154 ± 206	0.47 ± 0.16	0.118 ⁿ⁼⁵ ± 0.031	73.6 ± 7.6
Young n=9	119*** ± 11	79*** ± 9	112* ± 20	671** ± 173	968** ± 281	1212* ⁿ⁼⁷ ± 448	1649*** ± 495	0.70*** ± 0.12	0.097 ⁿ⁼⁷ ± 0.025	71.9 ± 6.2

Significant differences between young and old * $p < 0.05$, ** $p < 0.025$, *** $p < 0.005$

running and box stepping experiments described in Chapter V.

The older men had a markedly slower twitch response with significantly longer TPT ($p < 0.005$) and $1/2$ RT ($p < 0.005$). P_t was significantly lower in the elderly subjects ($p < 0.05$) and averaged 79% of that of the young subjects. Evoked tetanic and voluntary responses were all significantly lower in the elderly men averaging 77%, 73%, 69% and 70% of those of the young men for P_{010} , P_{020} , P_{050} and MVC respectively. There was no significant difference between the body weights of the two groups. The progressively greater fall in the force generated by the older men relative to the young men, with increasing stimulus frequency, did not result in a significant change in the twitch to tetanus (P_{050}) ratio ($p < 0.1$).

Comparison of the fatigue indices of the two groups showed the elderly to be significantly ($p < 0.005$) more fatiguable than the young when subjected to the standard stimulus regime. Typically the elderly subjects developed a tonus during the latter stages of the fatigue test, indicated by a raised baseline and merger of successive contractions, Fig. 4.1.

Discussion

From the results presented in Table 4.1 there is clear evidence that maximal twitch responses can be measured on the first visit to the laboratory in the elderly. Further these responses can be reliably reproduced on subsequent occasions and no habituation period is required. Supra-maximal tetanic stimulation at 10 and 20 Hz is more difficult to achieve but with care and patience all subjects were habituated to

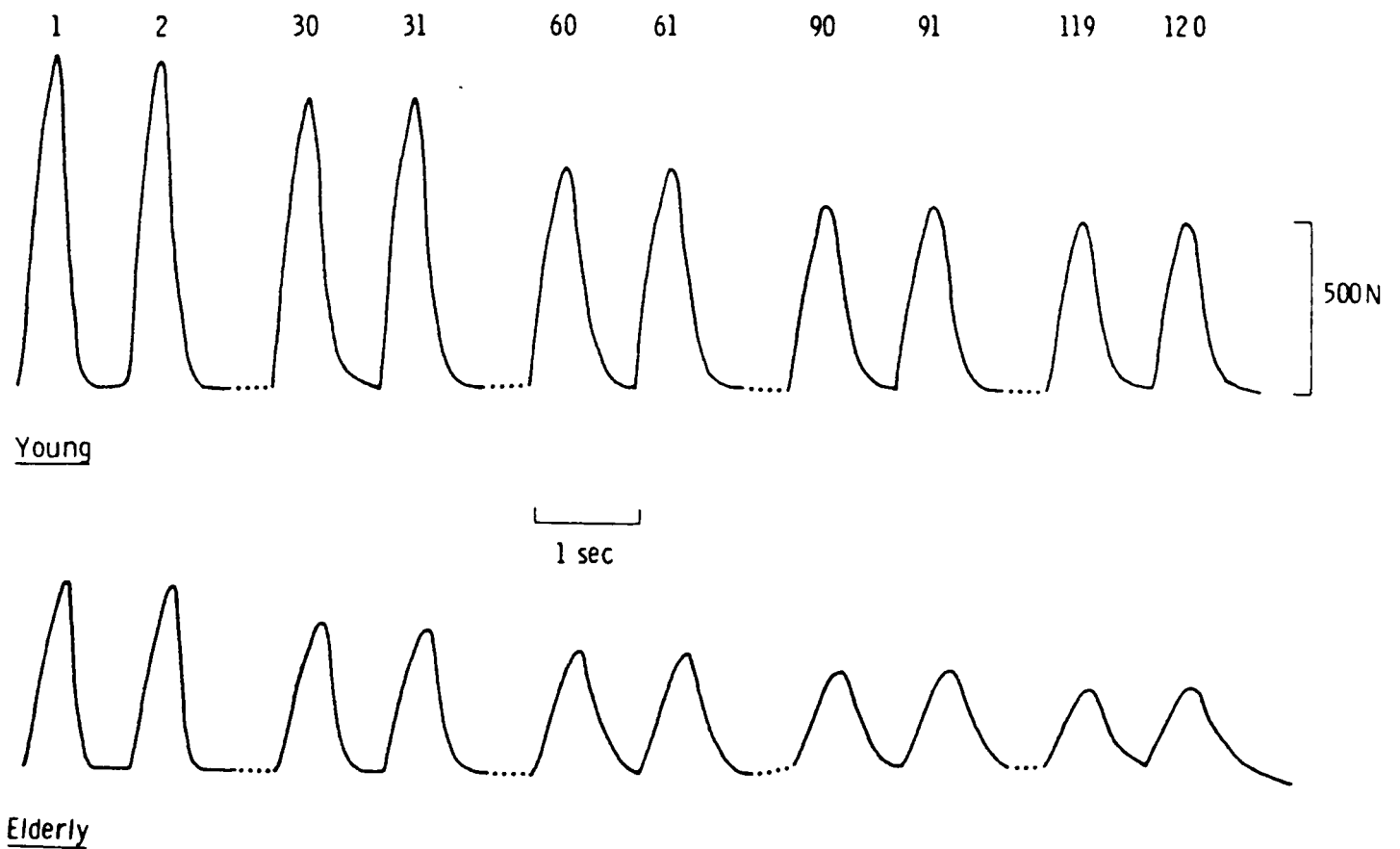


Figure 4.1.

Typical original records of fatigue tests in young and elderly triceps surae. Note the merger of successive contractions and raised baseline in the elderly subject towards the end of the test. The numbers above each contraction indicate the cycle number.

stimulation at this level after 2-3 visits to the laboratory. It was found that stimulation at higher frequencies was not acceptable to all subjects but five out of thirteen elderly men were tolerant of supra-maximal 50 Hz stimulation after 3 visits to the laboratory. Maximum voluntary contractions, measured repeatedly show that a period of habituation is required before reproducible measurements are obtained. This is worthy of note when testing strength in elderly subjects, and could lead to an underestimate if only a single set of measurements are made. The increase in MVC between visits 1 and 2 is unlikely to be due to hypertrophy because the intervals between visits for each subject varied from 1 to 4 weeks and because further improvements were not apparent over the subsequent visits. This implies that psychological or neural factors were responsible for the improvement (Ikai and Steinhaus, 1961; McDonagh et al, 1983; Davies et al, 1985).

Since there were no significant differences between any of the parameters measured for the third time and on subsequent occasions the values obtained on this visit, when all subjects tolerated supra-maximal tetanic stimulation, have been used in Table 4.2.

There are a number of significant differences between young and old triceps surae. The elderly subjects were, weaker than the young when both evoked and voluntary contractions were compared. Reductions in $P_{0.50}$ and MVC were similar, 31% and 30% respectively, indicating that after habituation to the required procedure, MVC is a reliable indicator of strength in well motivated 70 year old subjects. It is worthy of note that

Tzankoff and Norris (1977) showed a similar percentage fall in total muscle mass between the ages of 20 and 70 years, using 24 hours creatinine excretion as an index of muscle mass. This could be taken as evidence that the Triceps Surae are not preferentially affected by muscle wasting. However, the elderly men in the present study may have been much stronger in their youth than the young control subjects and therefore the actual reduction in their strength may have been much larger.

Since the body weights of the elderly men were no different from the young men but their maximum Triceps Surae strength was 30% lower, the relative load on this muscle group must be much higher during activity. This may be expected to affect the contractile properties of the muscle, in much the same way as that caused by excessive use in the slow twitch cat and rabbit soleus (Jewell and Zaimis, 1954; Vrbová, 1963). In both animals the time course of the isometric twitch contraction became prolonged when loading was increased following removal of the gastrocnemius. The finding of a progressive increase in the weakness of the 70 year old men relative to the young, as stimulus frequency was increased, shows that there has been a shift of the frequency response curve to the left. This is a feature of a slower contracting muscle and indeed mean twitch TPT and $1/2$ RT were found to be significantly increased by 24% and 23% respectively in the elderly subjects. The combined effect of increased TPT and $1/2$ RT is a prolongation of total contraction time (Fig. 4.2). A larger change in the same direction was found in twitches of elderly extensor hallucis brevis (E.H.B.) by Campbell et al (1973) when mean TPT and $1/2$ -

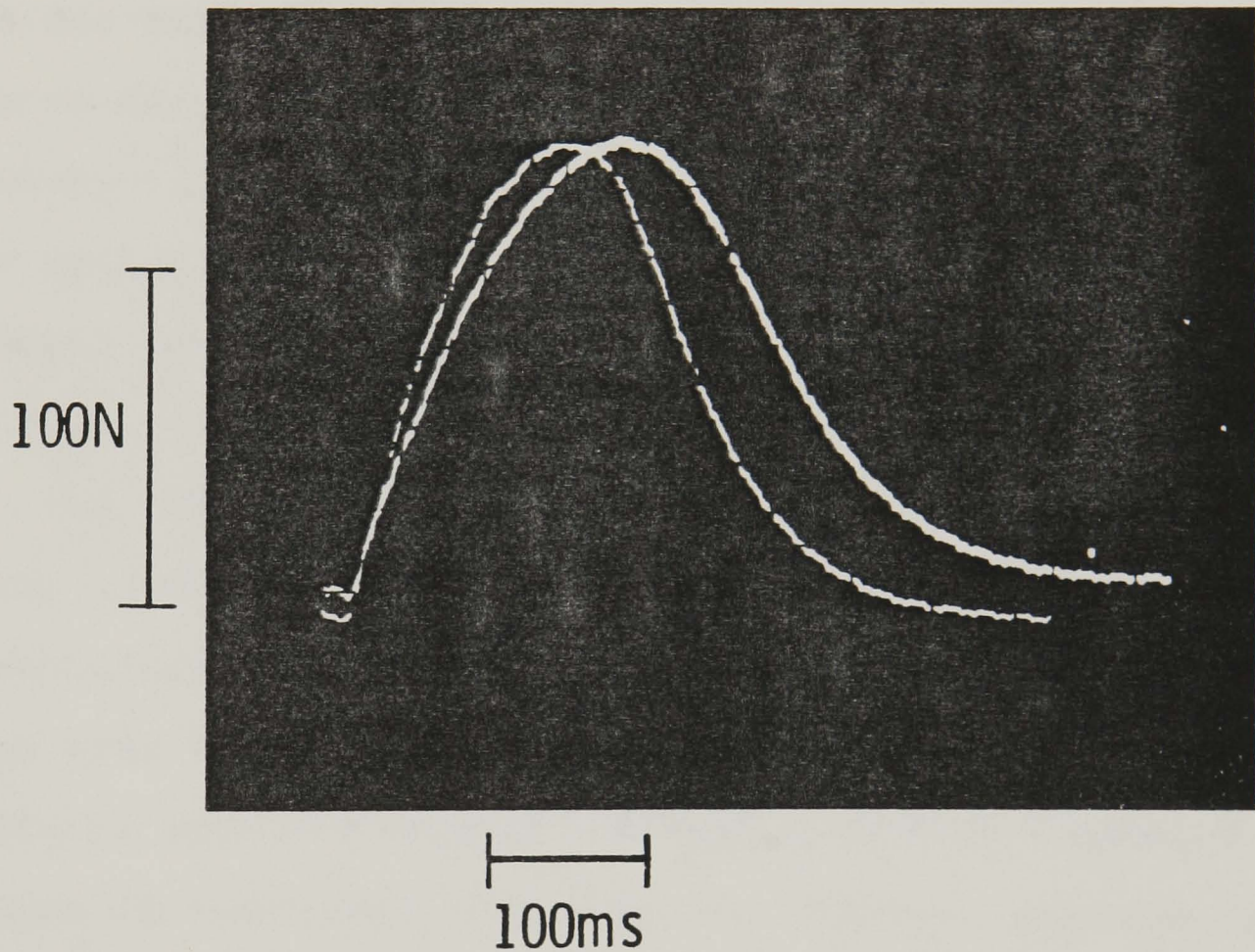


Figure 4.2.

Typical records of maximal twitch responses from a 70 and 20 year old man (the young man has the faster contraction). Note the baselines are offset.

RT were increased by 45% and 106% respectively in subjects between 60 and 96 years of age. The control subjects were aged 3-58 years previously shown to have no age related changes in twitch time course. It is interesting that two muscle groups of widely differing twitch time courses in control subjects, TPT 64 msec for EHB and 119 msec for the triceps surae, should both slow in old age. Further, this implies that the change is unlikely to be brought about simply by preferential 'ageing' of fast twitch motor units in either muscle, as was suggested by Campbell et al (1973) as one possible explanation. The argument for this statement is as follows.

The triceps surae are made up predominantly of slow twitch fibres (Johnson et al, 1973; Edgerton et al, 1975a). Nevertheless if there was a relative decline of fast twitch fibre area in the elderly a change in the twitch time course should be apparent (Biscoe and Taylor, 1967). Indeed a decrease in type II fibre area (fast twitch) has been reported in elderly gastrocnemius by Tomonaga (1977). Under such conditions twitch TPT would be expected to increase due to the loss of force generated early in the twitch by the reduced area of faster fibres. However, total twitch time course should not alter, as the force generated by the slower fibres which dominate the latter part of the twitch would be unaffected. Clearly this mechanism does not explain the increase in 1/2 RT and total contraction time seen in the elderly triceps surae nor the much larger prolongation of the twitch time course in the E.H.B.. Slowing of the twitch in both muscles must involve some change in the time course of contraction of individual muscle fibres.

As already discussed the availability of a smaller amount of muscle to move a body weight equal to that of a young subject, would be expected to cause just such a change over a period of time.

It has been shown in cat and human gastrocnemius (Burke et al, 1973; Garnett et al, 1979) that slow twitch motor units have a high resistance to fatigue when repeatedly stimulated by short tetanic trains of stimuli. A muscle group such as the triceps surae with a predominance of slow twitch fibres would therefore also be expected to show a high resistance to fatigue. In the young subjects a mean fatigue index of 0.70 confirms this expectation. However, it is something of a paradox that the elderly subjects with a significantly slower twitch than the young are significantly more fatiguable when subjected to the same fatigue test. One interpretation of this finding is that elderly slow twitch muscle is inherently more fatiguable than young but this seems unlikely. Grimby et al (1982) reported that the anaerobic and aerobic metabolic capacity of the remaining muscle mass of 78-81 year old quadriceps and biceps brachii was comparable to that of 20-48 year olds. In addition Taylor et al (1984) using N.M.R. spectroscopy on the forearm muscles of 70-80 year olds concluded that the energetics of human skeletal muscle are not altered by the ageing process. This view is further supported by the work of Larsson and Karlsson (1978) in 22-65 year olds and Aniansson et al (1978) in 70 year olds who showed that endurance, as measured by holding 50% MVC was maintained or even increased in elderly quadriceps. Dynamic endurance was also reported to be unaffected by age in

these experiments, but the measurement of peak rather than angle specific torque using a cybex dynamometer makes these results questionable (see Thomas et al, 1987).

A more likely explanation for the paradox of slower but more fatiguable elderly muscle can be given when other factors are taken into account. In the single motor unit studies of Burke et al (1973) and Garnett et al (1979) blood supply to the active muscle fibres was unlikely to be seriously compromised because only small forces were generated which could not cause an appreciable rise in intramuscular pressure. However, investigations of the relationship between force generation and blood flow in whole human leg muscles have shown circulation to be occluded at forces greater than 20% of MVC (Barcroft and Millen, 1939, Edwards et al 1972b). Since the slow twitch fatigue-resistant motor unit relies heavily on oxidative processes to produce the energy required for contraction (Burke et al, 1973; Garnett et al, 1979), a reduction in blood supply will reduce fatigue resistance and produce a lower fatigue index. One could predict that total ischaemia would result in a fatigue index of zero in a slow motor unit, and if the blood supply is partially occluded, a value between 0 and 1. It may be that the slower relaxation of elderly muscle, from a peak evoked force of between 40 and 50% MVC, restricts blood flow for a slightly longer portion of each cycle during the fatigue test. Indeed during the test relaxation becomes so prolonged in the elderly that after 60 contractions full relaxation does not take place between contractions and a 'tonus' develops (Fig. 4.1).

2 - Fatigue

Introduction

In the light of reported unchanging metabolic capacity and energetics in ageing muscle (Grimby et al, 1982; Taylor et al, 1984) the finding of a significantly lower fatigue index in elderly men, as compared to young men was surprising. This was further amplified by the prolonged twitch contraction time of the elderly subjects which would be expected of muscle with a very high proportion of slow twitch, fatigue resistant fibres (Burke et al, 1973; Garnett et al, 1979). The influence of blood flow on fatiguability in slow muscle has already been discussed as has the hypothesis that restricted blood flow accounts for the greater fatigue of the elderly triceps surae. Experimentally the importance of blood flow could be demonstrated by occlusion of the blood vessels which supply the lower leg. This can be readily achieved by inflation of a pressure cuff around the thigh. Under these ischaemic conditions force evoked during the elderly fatigue test would be expected to fall towards zero over the 2 min period. To be sure that the loss of force could not be ascribed to failure of excitation during the ischaemic fatigue test it is necessary to monitor the synchronous muscle action potential evoked by each supramaximal stimulus. If this regime was also applied to young muscle it would then be possible to compare the fatiguability of elderly and young slow twitch muscle under truly identical experimental conditions. Finally, if the lower fatigue index of the elderly under control conditions was related to slower relaxation between tetani then increasing the interval between

the 120 stimulus trains would allow more time for relaxation, better perfusion and so possibly greater fatigue resistance.

Methods

A series of experiments were carried out on a group of eight elderly men mean age 68.5 ± 5.4 years (range 60-77) four of whom took part in the previously described study. In all eight men control fatigue tests were performed as already described using a supramaximal stimulating voltage. On a different occasion the fatigue test was again performed but this time circulation in the lower leg was occluded using a thigh cuff containing a 50 x 18 cm bladder which was placed around the upper leg. This procedure did not interfere with measurement of force by the dynamometer. The cuff was inflated to a pressure of 200 mmHg (27 KPa) just prior to the start of the test and maintained at this pressure throughout, it was released immediately after the last stimulus ended making a total occlusion time < 2.5 min. Occlusion of the circulation in the lower leg was confirmed by the disappearance of the posterior tibial pulse at the ankle as the cuff was inflated. Control and ischaemic fatigue tests were also performed on one young (20 year old) subject, so that the effects of circulatory occlusion on a fatigue resistant muscle could be observed for comparison.

In a small group of elderly subjects muscle excitation was monitored during control and ischaemic fatigue tests. The synchronus muscle action potential was recorded from the skin above the surface of the soleus using a skin mounted preamplifier described by Johnson et al (1977). The stimulus

artifact was cancelled out using the circuit shown in Fig. 4.3 which was based on that of Hultman and Sjöholm (1983).

Finally, in a separate set of experiments using two elderly men the influence of relaxation time on fatiguability was investigated. In order to allow the elderly muscle time to relax fully between tetanic contractions during the fatigue test the interval between stimulus trains was increased. The normal fatigue test used a stimulus train of 300 msec repeated once per second, a total 'cycle length' of 1 second. In these experiments cycle lengths of 1.3, 2.3, 3.3, 4.3 and 5.3. secs were used. Regardless of the cycle length used each fatigue test carried out contained 120 stimulus trains lasting 300 msec at 20 Hz. The tests were carried out in succession on both subjects starting with the longest cycle length of 5.3 sec. A 7 minute rest period was given between tests. For comparative purposes the same protocol was applied to a young man known to have a normal fatigue index close to 1.0. It was thought the cycle length changes would produce little change in the fatiguability of this subject and he would act as a control.

Results

Circulatory occlusion resulted in a pronounced change in the fatiguability of the elderly triceps surae. Under control conditions mean force in the eight elderly men fell from 466 ± 143 to 208 ± 78 (N) while with circulation occluded force dropped from 434 ± 144 to 45 ± 26 (N), during the fatigue test. The slight difference in force at the start of the fatigue test under the two conditions was not significant ($p > 0.05$). Thus

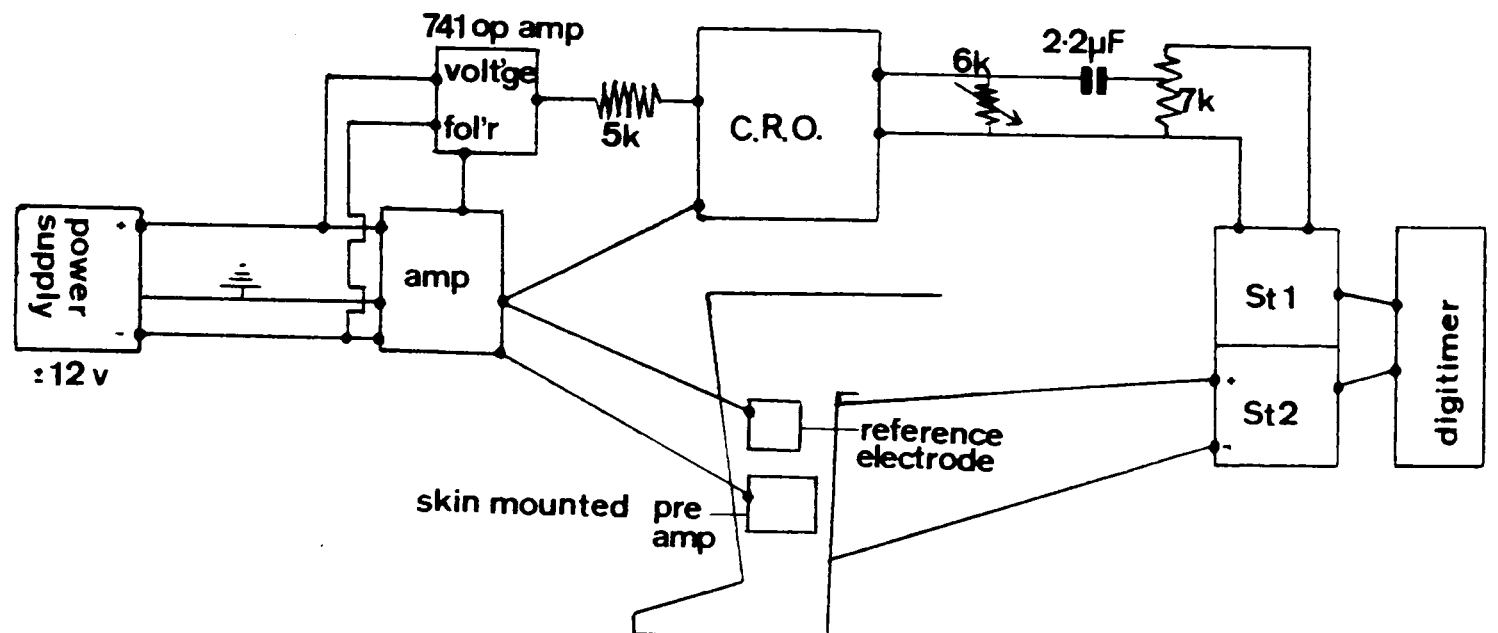


Figure 4.3.

Circuit used to balance the stimulus artifact during recording of synchronous muscle action potentials.

ischaemia resulted in a significant reduction in the fatigue index from a mean control value of 0.47 ± 0.16 to a value of 0.11 ± 0.03 ($p < 0.001$) Fig. 4.4. Indeed the absolute force generated during the ischaemic fatigue test became significantly different from that generated in the control test after only 30 sec ($p < 0.05$).

In the young subject measured under control conditions on six occasions force fell from a mean of 941 ± 45 to 578 ± 38 N while with circulation occluded it fell from a mean of 891 ± 17 to 100 ± 15 N. Thus his fatigue index changed from a control value of 0.59 to 0.11 with ischaemia.

In the group of elderly subjects in whom muscle excitation was monitored the fatigue index fell from a control value of 0.41 ± 0.19 to 0.10 ± 0.03 ($n=4$) with circulatory occlusion. During neither control nor ischaemic fatigue tests was there any evidence of excitation failure as judged by muscle action potential amplitude or duration. Figure 4.5 shows the relationship between evoked force relative to initial force, and amplitude of the last muscle action potential of each stimulus train relative to that found at the end of the first train of the test. Original records of muscle action potentials evoked by supramaximal stimulation under control and ischaemic conditions are shown in Figures 4.6 and 4.7 respectively. The tendency for amplitude to increase during the stimulus train, particularly at the start of each fatigue test was unaffected by circulatory occlusion. Similarly the slight rise in amplitude during the course of the test was equally unaffected.

The effect of changing cycle length on the fatigue indices

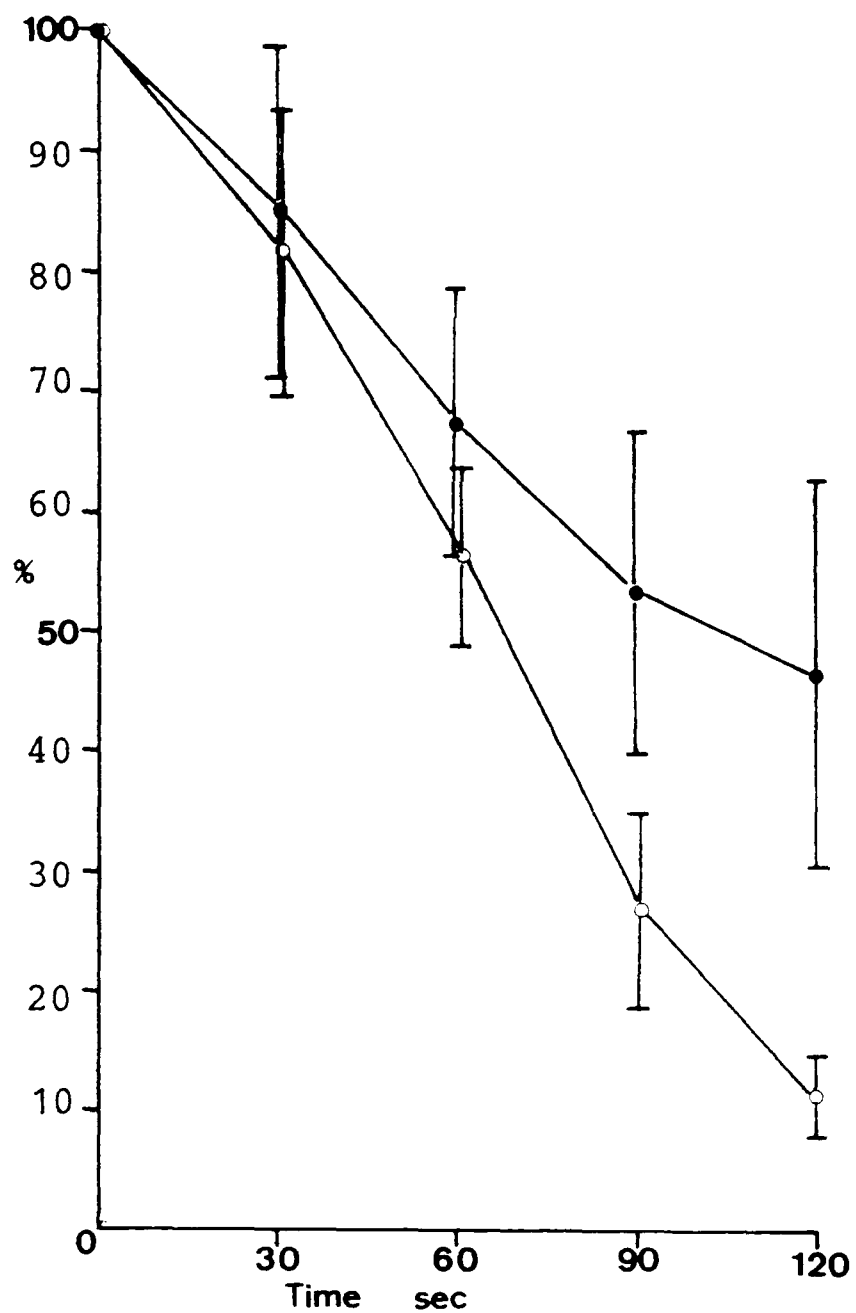


Figure 4.4.

Control (●) and Ischaemic (○) fatigue tests in the elderly triceps surae. Percentage of initial tetanic tension after 30, 60, 90 and 120 seconds of intermittent stimulation at 20 Hz.

Mean \pm S.D. n = 8.

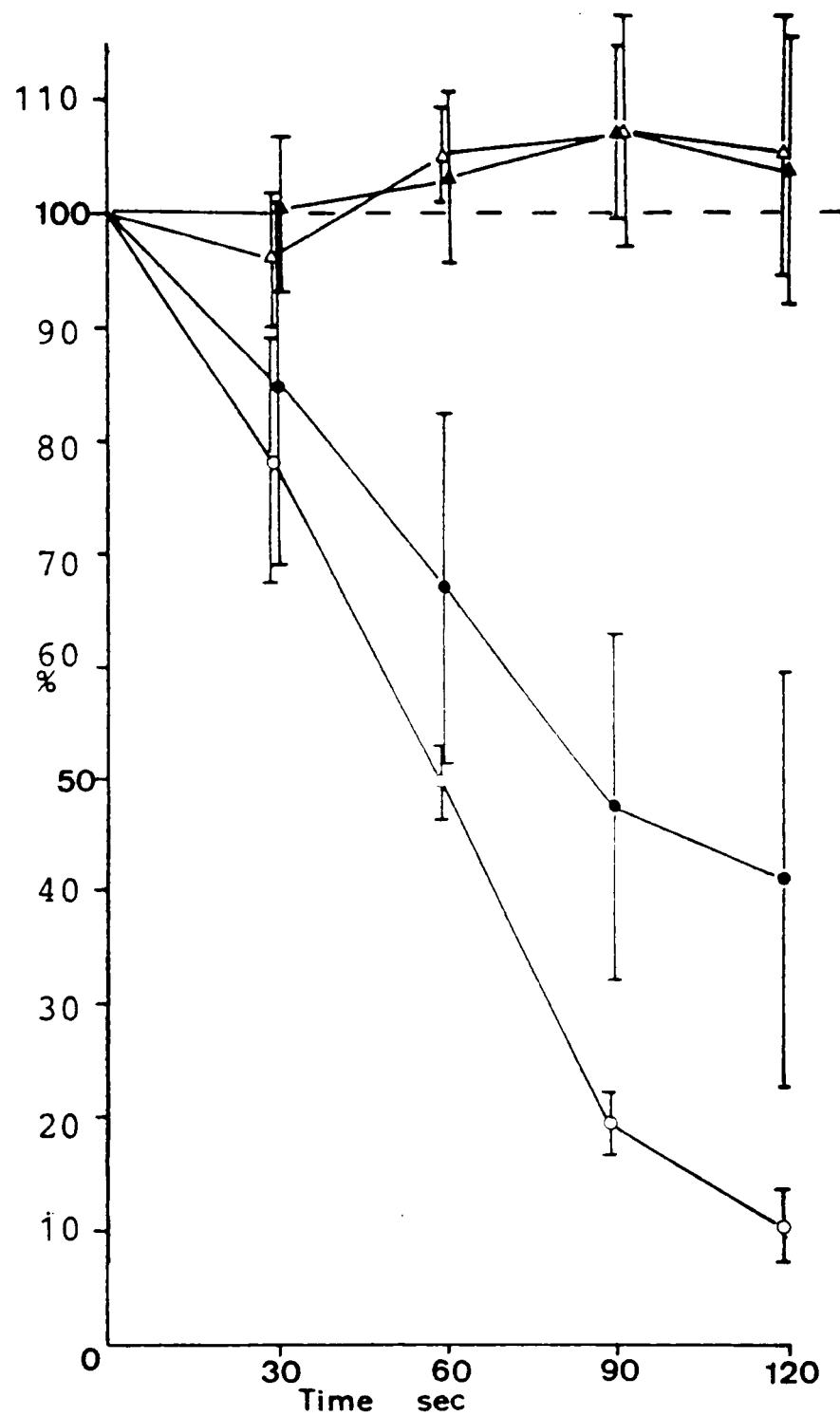


Figure 4.5.

Effect of ischaemia on force and the synchronous muscle action potential amplitude during 2 min. of intermittent stimulation. Data are expressed as a percentage of the initial response. Mean \pm S.D.

Control force (●) and 7th M-wave amplitude (▲)

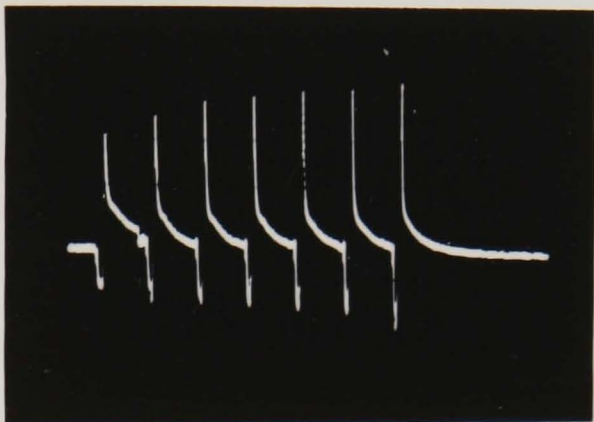
Ischaemic force (○) and 7th M-wave amplitude (△)

Figure 4.6.

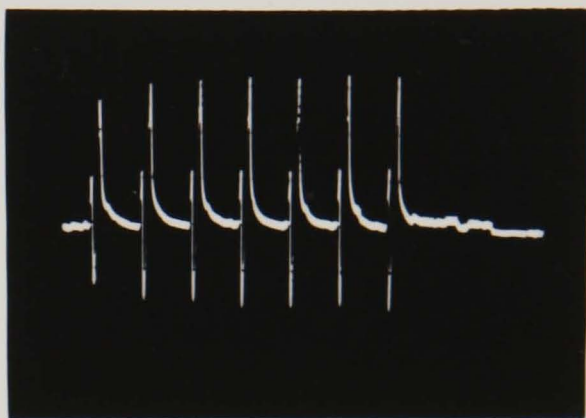
Typical synchronous muscle action potentials recorded during a control fatigue test performed on an elderly subject. Records are of the 1st, 30th, 60th, 90th and 120th stimulus trains.

Note that the first SAP of the 120th train was not recorded.

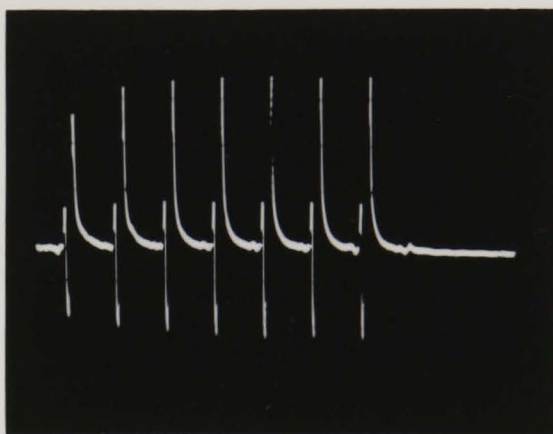
1



30



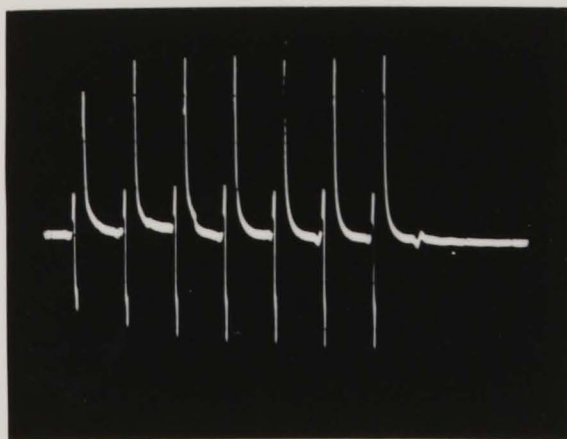
60



40
mv

100ms

90



120

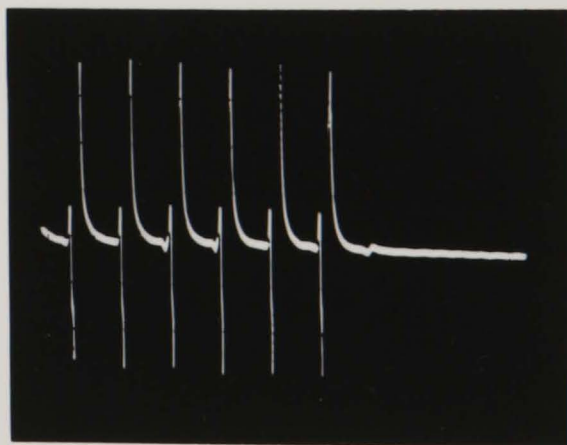


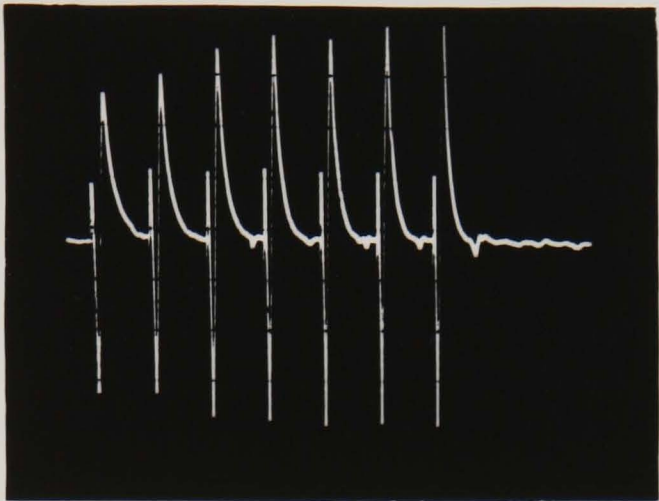
Figure 4.7.

Typical synchronous muscle action potentials recorded during an ischaemic fatigue test performed on another elderly subject.

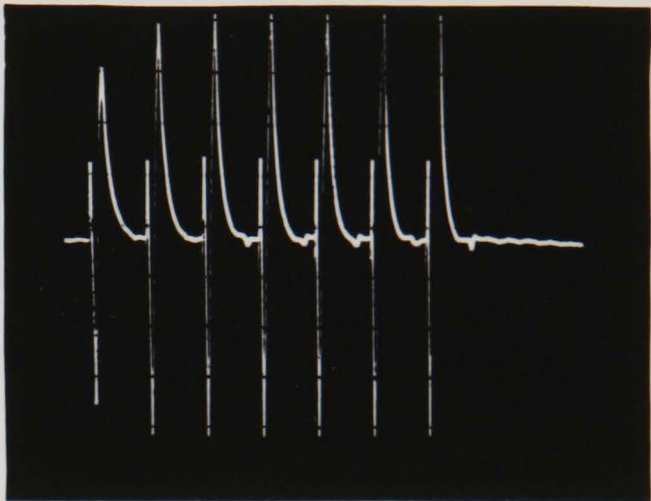
Stimulus trains are numbered as in Fig. 4.6.

Note that two SAP's at the start of the 60th train were not recorded.

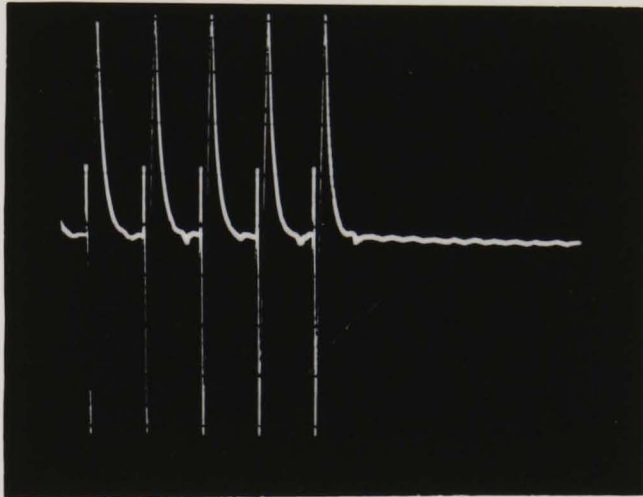
1



30



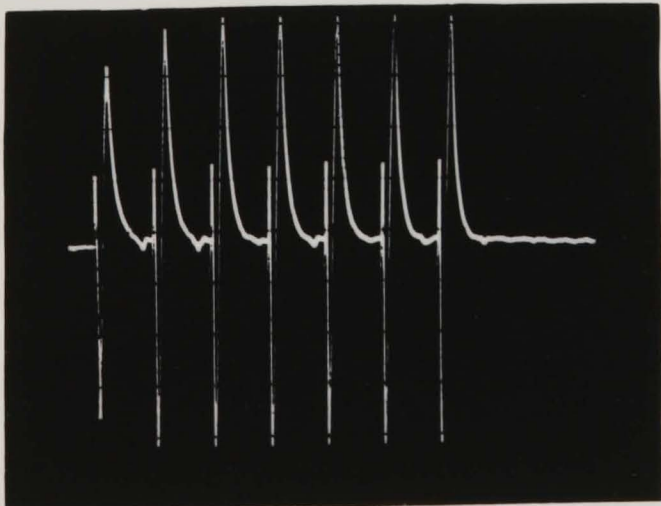
60



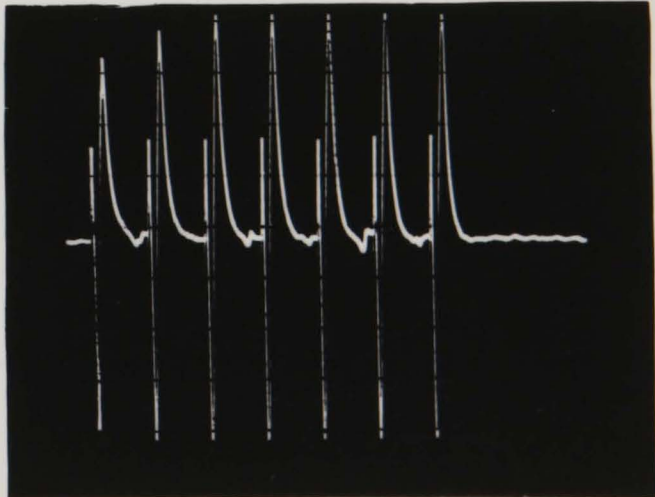
20
mv

100 ms

90



120



of the two elderly men and one young man is shown in Figure 4.8. At cycle lengths between 5.3 and 2.3 sec the tetanic response was potentiated, after a small number of cycles, resulting in an increase in the fatigue index to over 1.0 in both young and old. Reduction of the cycle length to 1.3 sec resulted in fatigue indices less than 1.0 in the elderly men while the young subject continued to demonstrate the potentiated response. The control values for the two elderly and one young man at a cycle length of 1.0 sec were 0.55, 0.46 and 0.95 respectively. Table 4.3. shows the absolute forces generated by the three subjects at the beginning and end of each fatigue test. It indicates that the protocol used did not alter the starting force in any of the subjects.

Discussion

Total occlusion of circulation in the lower leg of eight elderly men significantly reduced the fatigue index of the triceps surae to a mean value of 0.11 ± 0.03 from a control value of 0.47 ± 0.16 ($p < 0.01$) Fig. 4.4. This consistent response is as predicted (see page 97) for a muscle group with a predominance of slow twitch fibres, heavily dependent on oxidative metabolism. Under these ischaemic conditions the ATP required for contraction can only come from anaerobic metabolism and limited aerobic metabolism utilising oxygen stored in the tissues. There is evidence in the literature to suggest that the metabolic capacity (Orlander et al, 1978; Anian^{II}sson et al, 1981; Grimby et al, 1982) and energetics (Taylor et al, 1984) of the remaining muscle mass in the elderly is comparable to that

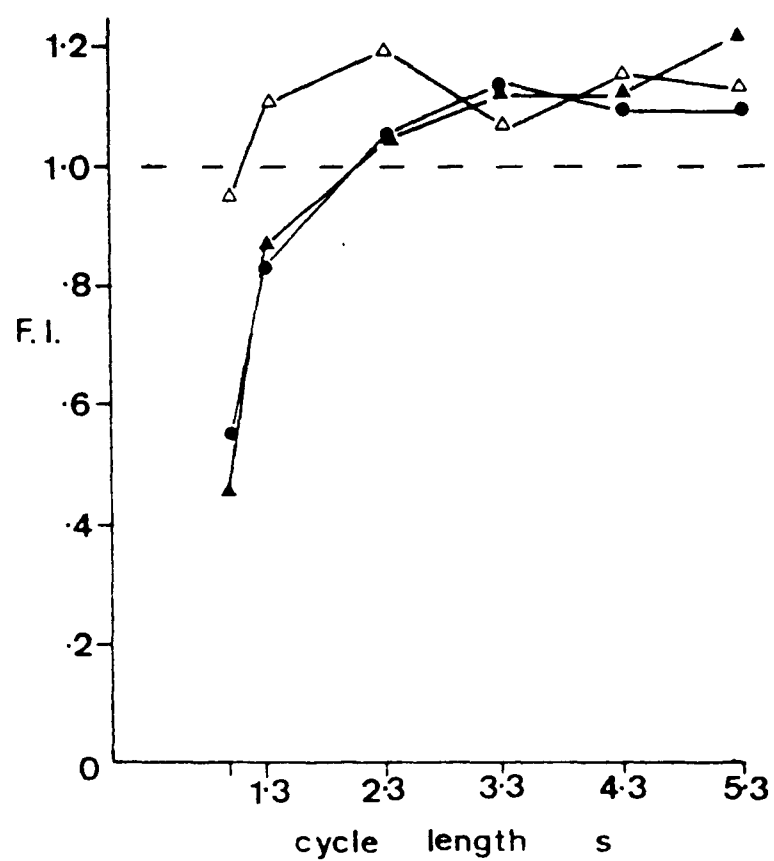


Figure 4.8.

The effects of changing cycle length on the fatigue index in 2 elderly men (▲,●) and 1 young adult (Δ). The results of 6 fatigue tests on each subject are shown. Each test comprised 120 cycles containing 30 msec of 20 Hz stimulation. The cycle lengths used were 1.0, 1.3, 2.3, 3.3, 4.3 and 5.3 sec.

TABLE 4.3

The effects of increasing cycle length on the fatiguability of the triceps surae in two elderly men and one young male subject. Forces developed at the start and end of each test are shown. 120 contractions were performed in every test. The fatigue index, FI is the force developed during the 120th contraction divided by that developed during the 1st contraction.

	Cycle Length (s)		5.3	4.3	3.3	2.3	1.3
E L D E R L Y	Force (N)	1 120	325 355	325 355	318 362	325 340	303 251
	F.I.		1.09	1.09	1.14	1.05	0.83
	Force (N)	1 120	377 459	392 444	392 444	414 436	385 333
	F.I.		1.22	1.13	1.13	1.05	0.87
Y O U N G	Force (N)	1 120	734 832	762 875	804 861	776 931	762 846
	F.I.		1.13	1.15	1.07	1.20	1.11

of younger muscle. It is therefore perhaps not surprising that under identical experimental conditions of ischaemia there was little difference between the profiles of force loss during the fatigue test in the 8 elderly men and in the young subject measured for comparison. If the metabolic capacity of muscle is maintained in ageing as the present results suggests why, then does the elderly triceps surae fatigue more than the young in the standard test?

It could be suggested that the loss of force during the fatigue test in the elderly was caused by excitation failure. However, this would not appear to be so as the muscle action potential recorded during both control and ischaemic tests was unchanged, see Figs 4.5, 4.6. and 4.7. Thus even under conditions of ischaemia which might have lead to presynaptic failure of transmission in animal muscle (Krnjević and Miledi, 1958) intermittent supramaximal stimulation at low frequency was shown to excite elderly human muscle normally.

From previous Results (page 90) it was suggested that the lower fatigue index found in the elderly could be related to the slower relaxation rate of elderly muscle. Blood vessels might then be occluded for relatively longer during each cycle and thus restrict blood flow and oxygen transport to a greater extent. This effect could become more pronounced as relaxation slowed during the test. By increasing the cycle length it might then be possible to minimise the effects of interruption of the circulation, if indeed this was a limiting factor. Comparison of elderly muscle with young fatigue resistant muscle would then be made more fairly, as was the case when using total occlusion.

At cycle lengths of 2.3 sec and above there was no loss of force during 120 successive contractions in young or old subjects. In fact the evoked response was potentiated and this resulted in a fatigue index of over 1.0 (Fig. 4.8). Clearly, at these stimulus intervals old and young muscle behaved similarly as would be expected from the preceding discussion. The responses to shorter cycle lengths were however markedly different. In the young man, chosen for his high normal fatigue index, reduction of cycle length to 1.0 sec caused only a small (5%) loss of force during the 2 min test while the elderly men showed a loss of approximately 50% in the same time. The increase of cycle length by only 300 msec markedly reduced this force loss and caused the elderly fatigue indices to rise above 0.80. Since muscle blood flow was not measured during these experiments it is impossible to ascribe this change with any certainty solely to better perfusion but full relaxation between tetani could certainly take place in the extra 300 m sec available using the 1.3 sec cycle length.

Definitive resolution of this problem must await techniques to simultaneously measure intramuscular pressure, blood flow and force during evoked contractions in human muscle (Petrofsky and Lind, 1975a and 1975b, Edwards 1972, Petrofsky and Hendershot, 1984).

CHAPTER V

The Effects of Exercise

1 - Running

Introduction

In isolated frog muscle fibres Grabowski et al (1972) showed that twitch tension could be reduced by over 90% after repetitive low frequency stimulation. This reduction could not be accounted for by a failure of the contractile machinery as caffeine restored the twitch force. Neither could the slight change in overshoot of the action-potential seen under these circumstances explain the results as a similar alteration in the action-potential caused by changing the sodium content of the bathing medium did not affect the twitch. Recovery of the twitch required 1-2 hours and the cause which was "tentatively suggested" for the deficiency in excitation contraction coupling was a decline in the concentration of an 'activator of contraction' localised in some part of the tubular system. Edwards, et al (1977b) demonstrated relatively greater reductions in low frequency tetani than high, in fatigued human muscle and termed this 'low frequency fatigue', a similar mechanism to that postulated by Grabowski et al (1972) was thought to be the cause. Low frequency fatigue was demonstrated in adductor pollicis using maximal electrically evoked tetanic contractions following sustained stimulated and voluntary contractions performed under ischaemic conditions. Evidence was also presented which suggested that low frequency fatigue occurred in human quadriceps following dynamic exercise, again using electrically evoked tetanic contractions but the evidence was not as conclusive.

Three experiments were performed, two in which the same

subject pedalled on a bicycle ergometer and one in which another subject stepped on and off a low chair. After cycling at a progressively increased workload until exhaustion, or for 1 hour at approximately 60% $\dot{V}O_2$ max the force evoked by low (20 Hz) frequency stimulation was depressed relative to that produced by high (50 Hz) frequency stimulation, recovering over the next one and-a-half hours. Stepping for 15 min reduced the low frequency force for up to 24 hours. These experiments used a stimulating voltage which activated only a portion of the quadriceps group and produced forces between 15 - 30% of MVC. Using this technique the absolute forces measured before and after exercise could not be directly compared because the exact number and type of the muscle fibres activated under each condition was not known. Theoretically this could also affect the ratio of the forces produced by low and high frequency stimulation, the 20:50 ratio, which will depend on the frequency response relationships of the individual muscle fibres activated by the external stimulus. However it is claimed that the results obtained by submaximal stimulation do give a reliable estimate of the function of the whole muscle (Edwards and Newham, 1984). Consequently the results of the dynamic exercise experiments were interpreted as low frequency 'fatigue' of the quadriceps, and attributed to excitation-contraction uncoupling as demonstrated in the adductor pollicis in the same paper. Whether there was a high frequency effect was not known because pre- and post-exercise responses to 50 Hz stimulation could not be compared directly and neither could they be 'normalised' by comparison to another response.

The ability to stimulate the triceps surae supra-maximally and evoke maximal responses at frequencies up to 50 Hz in some subjects, would allow the direct comparison of absolute forces. In addition the twitch response, evoked by a single stimulus, can be reliably and reproducibly measured in this group unlike the quadriceps. This is potentially of great interest, as it should be a sensitive indicator of any failure of activation.

Using supramaximal twitch and tetanic stimulation it should then be possible to clearly identify weakness and fatigue induced by voluntary dynamic exercise in the human triceps surae.

In cycling studied by Edwards et al (1977b) body weight is carried by the machine and involves quadriceps much more than triceps surae but running on a treadmill involves raising and lowering the centre of gravity with every stride and in this the triceps surae are heavily involved. Running speed can be adjusted to require any percentage of maximal aerobic power ($\dot{V}O_2\text{max}$) which can be sustained for a given period as in cycling (Åstrand and Rodahl, 1977). The present experiments were designed to measure the effects of running on the absolute force generated by the triceps surae, in response to supramaximal stimulation, particularly that evoked at low frequencies.

Methods

Five male subjects, mean age 21.4 ± 1.6 years, height 182.9 ± 3.5 cm and weight 74.9 ± 5.8 kg were recruited from within the Queen's Medical Centre, Nottingham. Three were regular

participants in team sports and the other two were regular runners but not elite performers. All were habituated to the involved procedures over several visits to the laboratory prior to the experimental day, however two of the subjects were unable to tolerate supramaximal tetanic stimulation at 50 Hz. The apparatus and stimulation technique were as described in Chapter II section 1. In some of these experiments the actual voltage across the stimulating electrodes was measured using an oscilloscope, at each stimulating voltage used. Control measurements were made on the preferred leg of each subject, submaximal and maximal twitch and tetanic responses were recorded together with MVC's and the Fatigue Test. After one hour running at 7 mph 0% gradient on a motor driven treadmill the leg was remeasured and then again 90 min. post-exercise.

Results

A summary of the twitch response for the five subjects, mean \pm S.D., is shown in Table 5.1.

TPT was significantly reduced ($p < 0.05$) following exercise but had recovered 90 min after the end of the exercise period. $1/2$ RT was not significantly affected by running for one hour. Typically stimulus response curves showed one of two effects; those with no change in max P_t were shifted to the left post-exercise giving greater twitch force for a given submaximal voltage (Fig. 5.1.) and those where max P_t was reduced slightly post-exercise with no curve shift (Fig. 5.2). The mean reduction in P_t was not significant.

Table 5.2, shows the effects of running on the tetanic

TABLE 5.1.

Effects of 1 hr running at 7 mph 0% gradient on the maximal twitch characteristics of the triceps surae.

	TPT (msec)	1/2RT (msec)	Pt (N)
Control	121 ± 12.9	75 ± 8.8	110 ± 21.1
Post-exercise	109* ± 9.9	74 ± 6.2	104 ± 30.1
Recovery	127 ± 8.4	74 ± 7.8	114 ± 24.1

* $p < 0.05$

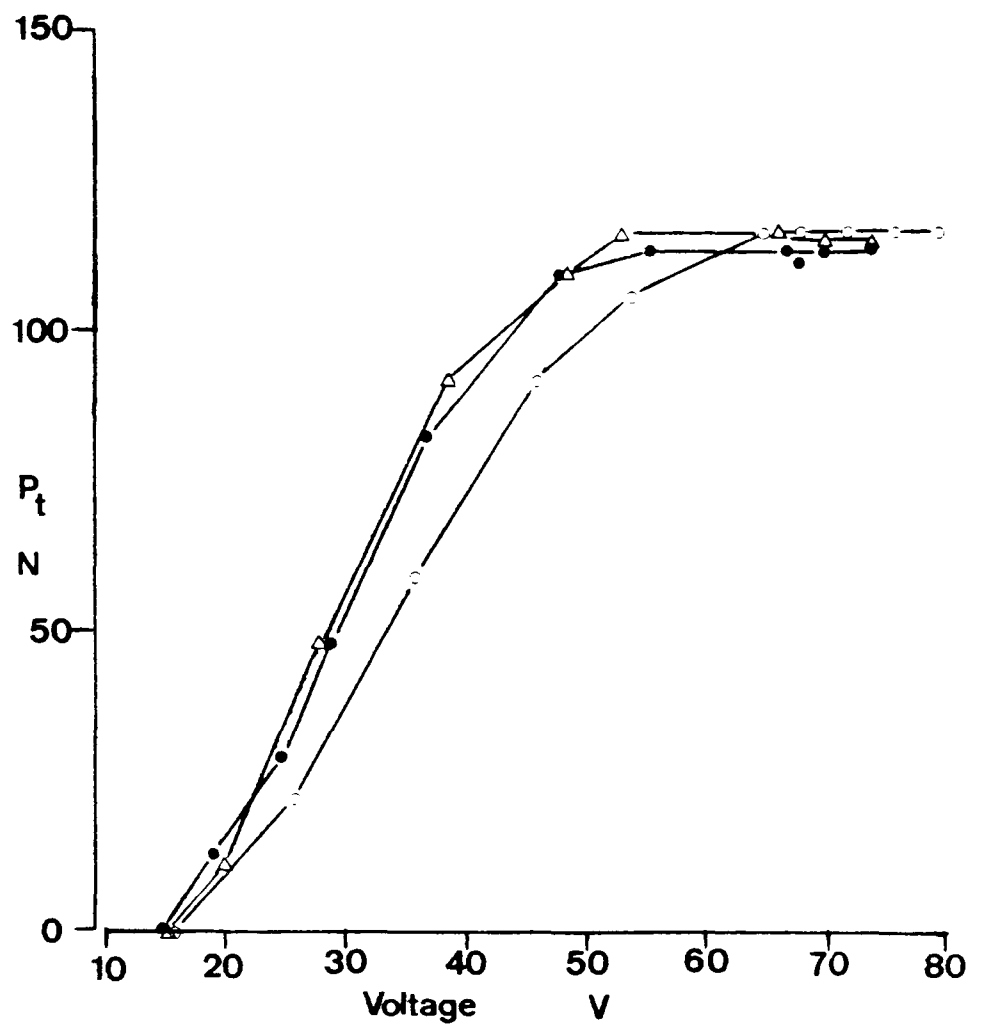


Figure 5.1.

Shift of the twitch stimulus response curve to the left following 1 hour of running.

Symbols; (o) control, (●), post-exercise, (Δ) 90 min recovery.

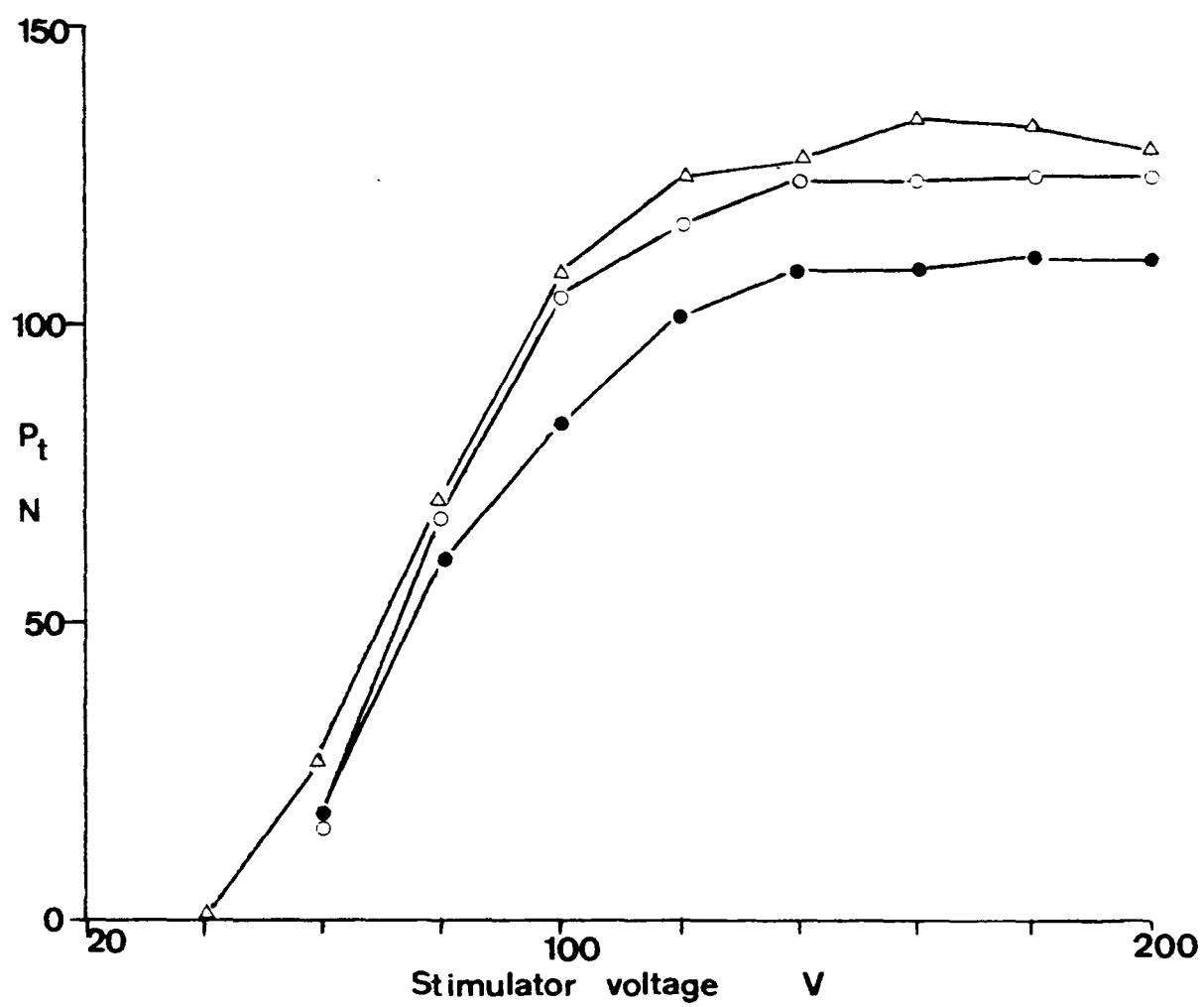


Figure 5.2.

Depression of P_t without a shift of the stimulus response curve following 1 hour of running.

Symbols; (o) control, (●), post-exercise, (Δ) 90 min recovery.

tensions at stimulus frequencies of 10, 20 and 50 Hz together with its effects on MVC and the Fatigue Test.

Post-exercise tetanic force at 10 Hz ranged from 1% greater than control to 23% less, the mean change for all five subjects being 8% less (NS), but at 20 Hz there was a statistically significant fall averaging 12% (range - 1% to - 22% $p < 0.05$). Two subjects were unable to tolerate maximal 50 Hz stimulation but in the three who could, force fell by an average of 12% (range - 2% to - 17%) (NS).

MVC decreased significantly ($p < 0.02$) following exercise in all subjects by between -3% and -13%, the mean value was -6%.

The force evoked by the first stimulus train of the Fatigue Test post-exercise was lower than under control conditions by an average of $13.6 \pm 5.3\%$ ($p < 0.01$) but the force produced by the 120th train was not significantly different from control. As a result the mean fatigue index was slightly increased post-exercise but this difference was not statistically significant.

Post-exercise tetanic stimulus response curves were shifted to the left of control curves in all subjects regardless of the degree of weakness produced. Fig. 5.3a shows the typical stimulus response curves at 20 and 50 Hz pre- and post-exercise for one subject. In this case no weakness at 20 Hz was apparent after exercise (note that the 50 Hz response had not reached maximum when discontinued at the subject's request). It can be seen that measurement of absolute force plus use of a constant submaximal stimulating voltage before and after exercise would lead to the false assumption that there was an increase in muscle force at both frequencies of stimulation after exercise.

TABLE 5.2.

Effects of 1 hr running at 7 mph 0% gradient on the maximal evoked force at frequencies of 10, 20 and 50 Hz, the maximal voluntary force and fatiguability of the triceps surae.

	P ₁₀ N	P ₂₀ N	P ₅₀ N	MVC N	FI
	n=3				
Control	740 ±176	1065 ±300	1388 ±554	1780 ±539	0.68 ±0.08
Post- exercise	679 ±222	942* ±170	1214 ±455	1669** ±548	0.75 ±0.11
Recovery	719 ±199	1035 ±295	1358 ±509	1744 ±534	0.70 ±0.10
	* p < 0.05				
	** p < 0.02				

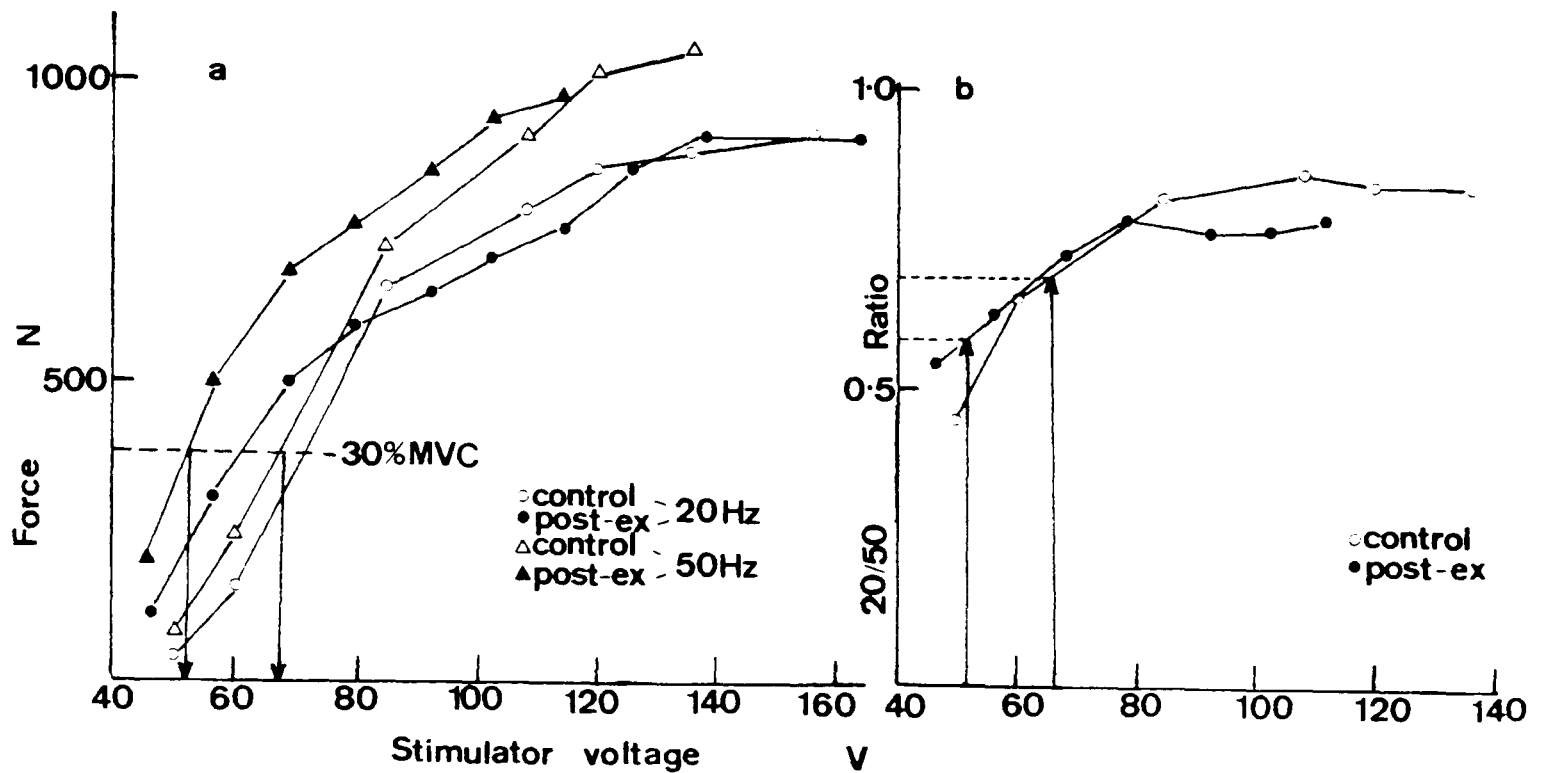


Figure 5.3.

(a) Typical stimulus curves for 20 and 50 Hz tetanic stimulation showing shift to the left after running for 1 hour. The dotted line indicates 30% control MVC while the solid vertical lines indicate the voltages required to evoke this force when stimulating at 50 Hz before and after exercise.

(b) The dependence of the 20/50 ratio on stimulus voltage before and after exercise. The vertical lines are those found in (a) required to evoke 30% MVC with 50 Hz stimulation. The horizontal dotted lines indicate the 20/50 ratio at these voltages.

The relationship of 20:50 ratio and voltage pre- and post-exercise is shown in Fig. 5.3b for the same subject. Typically both before and after exercise the ratio was found to be voltage dependent being lower at low stimulus intensities and increasing with stimulus voltage to a value at which it becomes essentially constant with increasing voltage. In this instance post-exercise low voltage stimulation gave a higher 20:50 ratio than control but in the other subjects there was considerable variation. In some the ratio was lower than control in others it was identical. These changes were not related to the absolute change in plateau force produced by exercise.

Discussion

The results of this investigation show that one hour of running at 7 mph has a small and short lasting effect on the contractile properties of the triceps surae. The decrease in twitch TPT by an average of 12 msec after exercise is of the same order as that seen following passive heating or a 15 minute active warm up (Chapter II). It is most likely that the cause of this effect is increased muscle temperature, associated with exercise on either or both of the two major processes responsible for the twitch characteristics (Bennett, 1984). These are released and re-uptake of Ca^{2+} by the sarcoplasmic reticulum (Brody, 1976) and the intrinsic speed of shortening of the muscle fibres governed by myosin ATPase activity (Bárány, 1967). Upon release of Ca^{2+} into the myoplasm and activation of the contractile process more rapid shortening would perhaps allow normal force development before faster Ca^{2+} re-uptake by

the sarcoplasmic reticulum stops the contraction. In this way the finding of a decreased TPT but unchanged P_t may be explained. Certainly there is no evidence of a failure of activation in response to a single stimulus following running, which would be expected if there was uncoupling of excitation and contraction (Edwards et al, 1977b).

The same mechanisms that caused the speeding up of the twitch would also be expected to increase the tetanic fusion frequency thereby contributing to the decreased force at lower stimulus frequencies. Passive heating caused a 15% reduction in P_{010} and active warm up, by running for 15 min to produce the same muscle temperature, caused a slightly greater fall of 18% (Chapter II). The changes in P_{010} after one hour running though not statistically significant were in this range and could therefore be accounted for by raised muscle temperature post-exercise. However, it must be remembered that P_{020} was unaffected after passive heating but was significantly reduced by 12% after active warm up, suggesting a different mechanism was responsible for this force loss. The reason could be genuine muscle weakness but if so the finding that the fall in P_{020} after one hour of running was still only 12% implies that the weakness does not become progressively worse in that time. The significant fall in MVC which is known to require high firing rates reaching 80-120/sec for short periods (Freund, 1983) and the reduced P_{050} values in the subjects able to tolerate supramaximal stimulation could not be due to raised muscle temperature as in both cases the contraction is fully fused. Overall these changes must then be indicative of a small degree

of muscle weakness which it appears is not confined to low stimulus frequencies, however recovery of this weakness is complete after 90 min. Though significantly weaker the triceps surae failed to show evidence of an increased fatiguability after exercise since the force evoked by the final train of stimuli in the Fatigue Test given post-exercise was not significantly different from that given under control conditions.

Clearly the finding of enhanced responses evoked by submaximal stimulation post-exercise (Fig. 5.3a) when maximal responses are reduced or unchanged highlights the unsuitability of using absolute forces, evoked by anything other than supra-maximal stimulation, for comparative purposes. Attempting to overcome this problem by use of a ratio of forces produced by 20 and 50 Hz stimulation also appears misleading as this ratio is voltage-dependent in the triceps surae both before and after exercise (Fig. 5.3b). The combination of enhanced submaximal responses and voltage dependent 20:50 ratio could then lead one to believe that specific low frequency (20 Hz) force failure had occurred. If in Fig. 5.3a a level of force equivalent to 30% MVC is used as the maximum 50 Hz response used before and after exercise, as described by Edwards et al (1977b) then several points arise. At the voltage required to evoke this response prior to exercise the 20:50 ratio is 0.69 (Fig. 5.3b) well below the value of 0.85 calculated at higher and maximal voltages. Following exercise because of the enhanced submaximal responses to stimulation a lower voltage is required to produce a 50 Hz tetanus equivalent to 30% control MVC, and the 20:50 ratio is

0.59. This could be taken as evidence of low frequency 'fatigue' but in fact the maximal 20 Hz response is identical to that pre-exercise.

The voltage dependence of the 20:50 ratio is probably due to the pattern of fibre recruitment with increasing stimulus voltage. Cable theory predicts that the larger the diameter of an axon the lower its threshold to an external stimulus applied through distant electrodes and the higher the axonal conduction velocity. Conversely the theory predicts that small fibres have a lower threshold current required to initiate an action potential through a direct application of current and this forms the basis of Hennemann's 'size principle' (1957). Taking the case of externally applied stimulation as the stimulus of the triceps surae, then it would seem logical that as current density increases and spreads, inwards large fast axons will be recruited before small. It has been shown (Appelberg et al, 1967) that the higher the conduction velocity of an axon the faster the contraction time of the muscle fibres it innervates and the larger the force the motor unit produces. These faster fibres require higher stimulus frequencies to become fully activated (Burke et al, 1973; Garnett et al, 1979) and consequently have a lower 20:50 ratio. Thus at lower stimulating voltages preferential recruitment of fast forceful motor units could produce a low 20:50 ratio. As the voltage is increased and spreads further into the muscle then this effect would continue at the boundaries of the stimulus. However with larger numbers of slower less forceful motor units also being recruited within the boundary of the stimulus the 20:50 ratio

would be expected to rise, the final value of the 20:50 ratio achieved depending on the relative fast and slow fibre areas. In the experimental situation the proximity of an external electrode to an axon or branch of an axon will also influence the recruitment of a motor unit. This same mechanism probably also explains the observation often made during these and other experiments of a faster twitch at very low stimulus intensities.

This investigation has demonstrated that submaximal stimulation of the triceps surae both before and after exercise produces results which could be misleading if taken as representative of the whole muscle group. It also showed that the weakness induced by running was small, (-12%) short lasting, (< 90 min) and could be demonstrated at a high (50 Hz) frequency of stimulation as well as a low (20 Hz) frequency. The use of an electrically evoked 'fatigue' test showed that although the initial force was reduced after exercise the fatiguability of the muscle, i.e. the ability to repeatedly produce a given force was not affected significantly.

These results do not provide strong evidence for low frequency fatigue in the triceps surae, following one hour of running. Primarily this is because there was no significant reduction in P_t and also the weakness observed following exercise was small and not confined to low frequency tetanic stimulation, there were in fact similar relative reductions at all frequencies. Excitation-contraction uncoupling may be expected to reduce P_t by at least as much as low frequency tetanic force and tetanic force should be progressively less affected as stimulus frequency is increased. A decrease in the

amount of activation per pulse which is the proposed mechanism of the phenomenon would have less effect on higher frequency stimulation, "because the total summated level of activation approaches the saturation level and a change in the amount of activation per pulse would have a smaller, or negligible, net effect" (Edwards et al, 1977b).

Perhaps the triceps surae are protected from this E-C uncoupling because of the predominance within the muscle of type I fibres (Gollnick et al, 1974c; Johnson et al, 1973; Edgerton et al, 1975a) with high oxidative capacity (Garnett et al, 1979). Kugelberg and Lindegren (1979) showed that motor units in rat tibialis anterior had a resistance to low frequency fatigue, induced by low frequency stimulation, which was directly correlated to the intensity of the activity of succinate dehydrogenase, a key oxidative enzyme within the fibres and Jami et al (1983) could find no E-C uncoupling in type S (slow twitch) fibres in cat peroneus tertius. Alternatively the aerobic nature of the exercise chosen may not provide the necessary conditions for low frequency fatigue. The experiments reported by Edwards et al (1977b) on human adductor pollicis and most of those showing long-lasting effects on quadriceps used isometric contractions performed under ischaemic conditions or in one instance box stepping.

It may be that some component of box stepping not present in running induces low frequency fatigue. Therefore further experiments were carried out involving box stepping.

2 - Box Stepping (i)

Introduction

In the first set of experiments a subject exercised using alternate, and then constant leg lead protocols.

Methods

In both experiments a subject stepped on and off a box, the height of which was adjusted to just below patellar height at a rate of 20 lifts of the body/min which allowed him to carry on for one hour at a similar oxygen cost to the running. The normal practice in such box-stepping experiments is to alternate the leading leg so that the lifting of the body is shared equally between both legs and this practice was followed in the first experiment. In the second experiment the subject led with the same leg throughout the exercise period.

Measurements of twitch, tetanic and maximum voluntary forces were made before and after exercise and during recovery after 2-3 hours and 21-22 hours in both legs.

Results

Alternate leg box stepping produced falls in maximum twitch tension in both legs post-exercise of -19% in the right leg and -12% in the left leg. As in the running experiments submaximal responses were enhanced. (Figure 5.4).

Falls in maximum tetanic tensions were greater than those of the twitch being 40% and 31% at 10 Hz and 22% and 26% at 20 Hz in the right and left legs respectively. Again submaximal

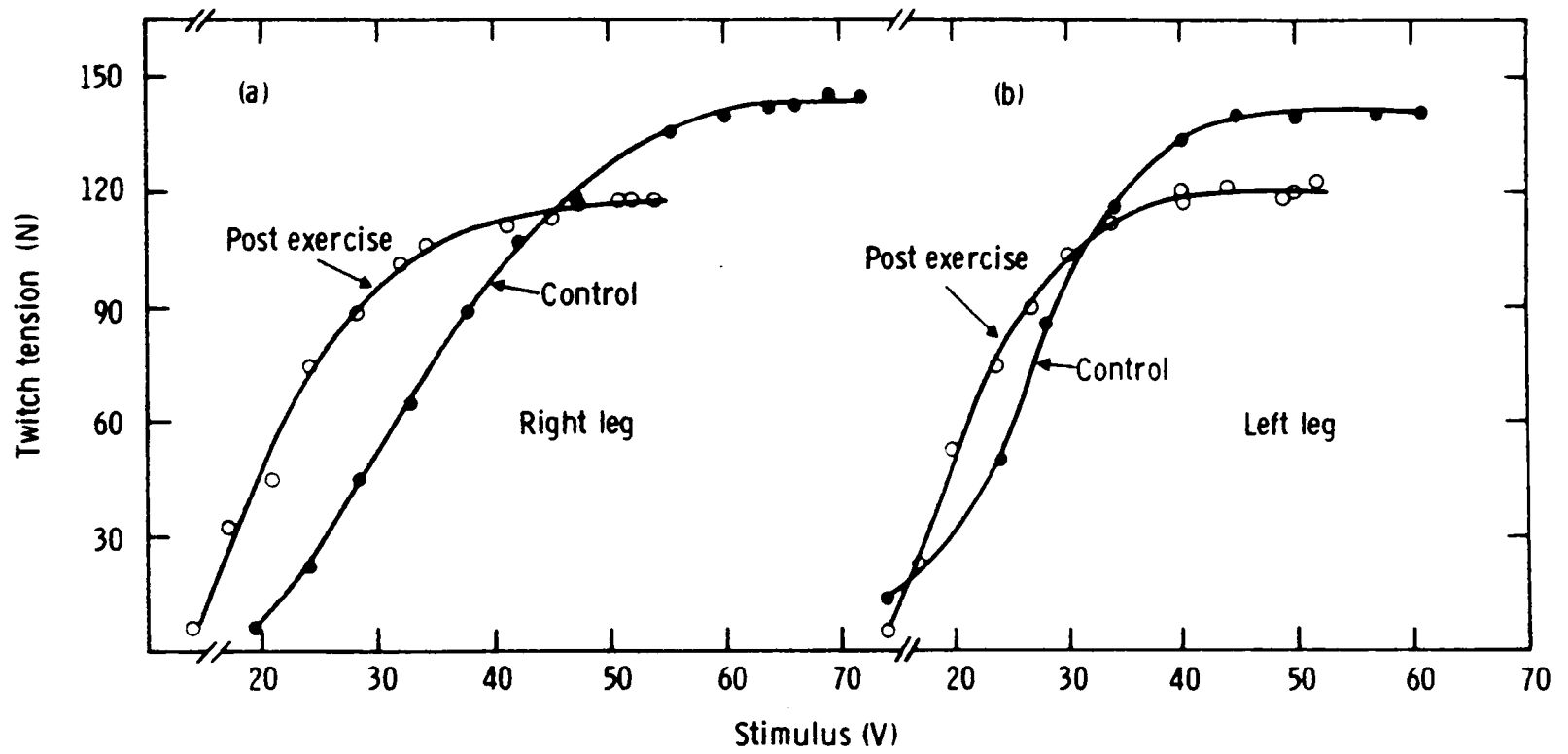


Figure 5.4.

The effect of 1 hour alternate leg lead box stepping on the twitch stimulus response curves of (a) the right and (b) the left leg.

responses were enhanced (Fig. 5.5). Unfortunately, maximal 50 Hz was not tolerated by the subject in these experiments. MVC was reduced by 17% and 4% in the right and left legs respectively. The results are summarised in Table 5.3 and show that recovery was slow, maximum tetanic tensions were still depressed 21-22 hours post-exercise though P_t had recovered by this time.

Figure 5.6 shows the effects of box stepping with a constant leading leg on the twitch responses from the two legs. In the concentric left leg, P_t was reduced by 6%, half the fall in this leg following alternate leg lead stepping. The right leg having performed eccentric contractions showed a fall in P_t of 24%, four times that of the left leg and in contrast to the concentric leg showed a reduced response at submaximal as well as supramaximal stimulating voltages.

Maximal 10 and 20 Hz tetanic responses in the concentric leg were reduced by only 7% and 9% respectively whereas the corresponding eccentric values showed a reduction of 55% and 51%. These dramatic decreases were not fully recovered after 24 hours when the maximum 10 Hz response was still reduced by 21% and the 20 Hz response by 11%. As with P_t in the eccentric leg submaximal P_{o10} and P_{o20} responses were depressed relative to control but in the concentric leg they were enhanced. MVC was little affected by the box-stepping in the concentric leg but was reduced by 23% in the eccentric leg and was still reduced by 7% after a 24 hour recovery period.

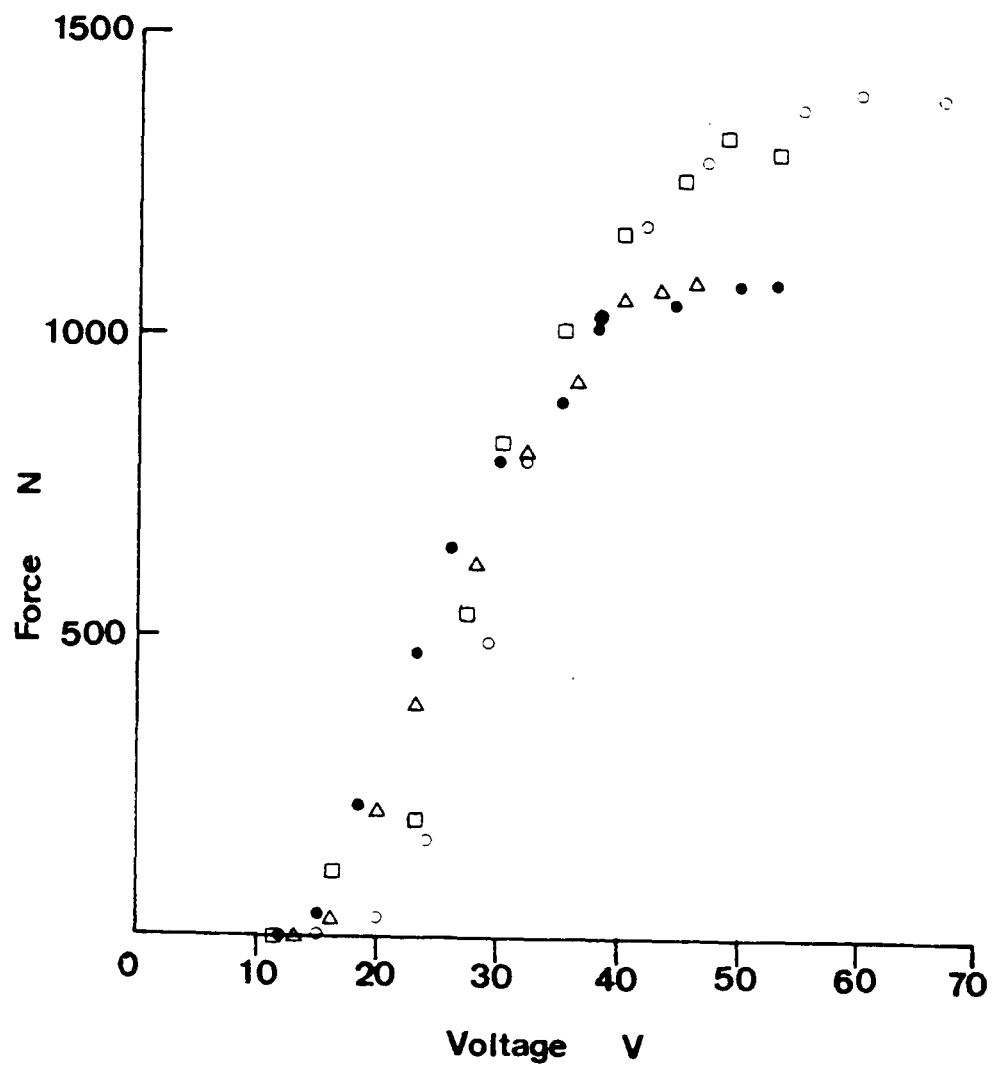


Figure 5.5.

20 Hz stimulus response curves from the right leg of a young male, following 1 hour alternate leg lead box stepping.

Symbols; (o) control, (●) post-exercise, (Δ) 2 hours and (□) 21 hours of recovery.

TABLE 5.3.

Effects of alternate leg lead box stepping on the contractile properties of the triceps surae in Right and Left Legs.

		TPT msec	1/2RT msec	Pt N	P _O 10 N	P _O 20 N	MVC N
R	Control	130	70	145	1023	1398	2384
l e g	Post- exercise	110	70	117	618	1089	1972
	+2 Hrs	110	70	127	692	1097	2286
	+21 Hrs	130	70	140	909	1319	2158
L	Control	130	70	141	986	1324	2050
l e g	Post- exercise	120	80	124	677	986	1962
	+2.5 Hrs	120	70	127	603	942	1903
	+22 Hrs	130	70	148	741	1045	1834

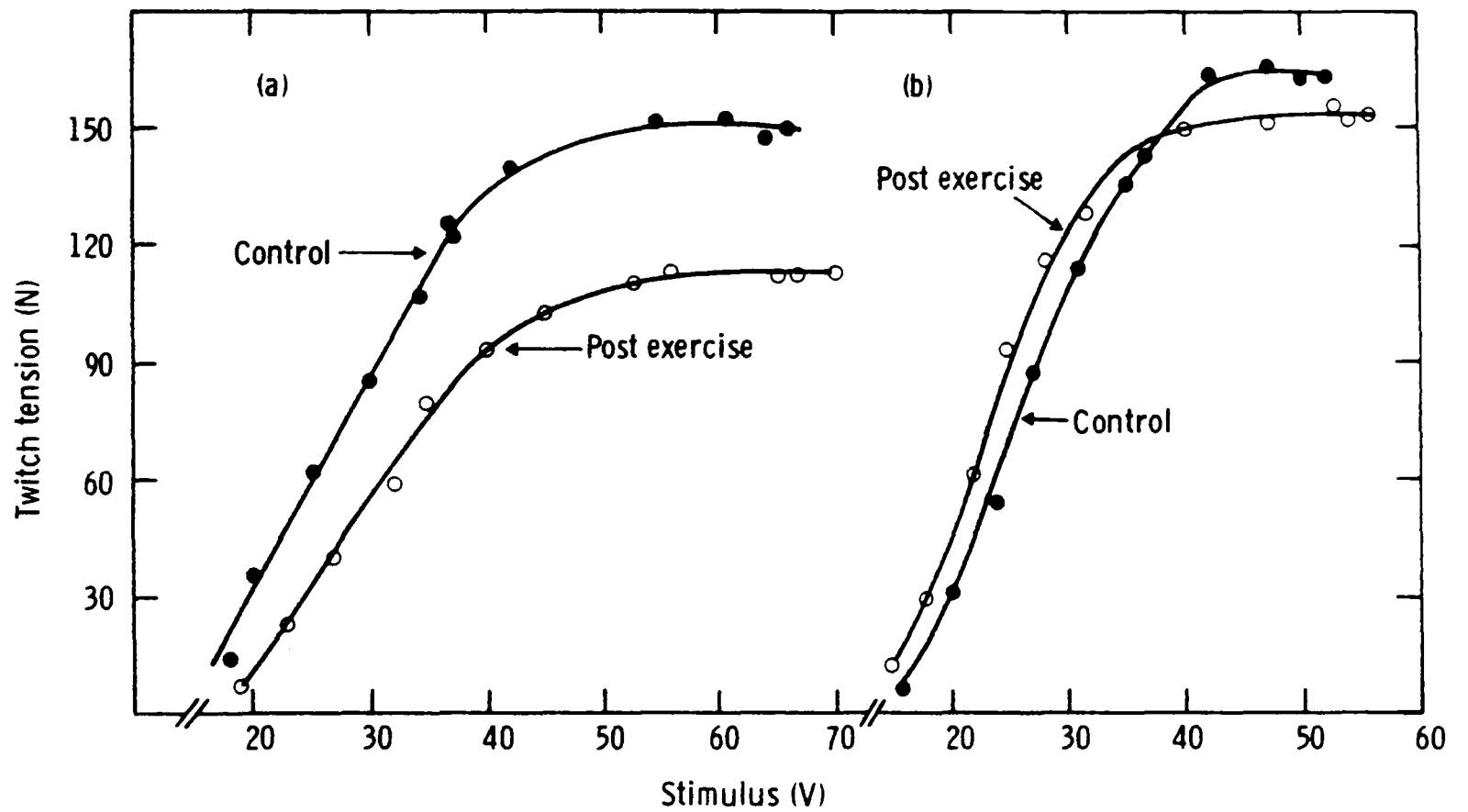


Figure 5.6.

The effects of 1 hour of right leg lead box stepping on the twitch stimulus response curves of (a) the right leg performing eccentric contractions and (b) the left leg performing concentric contractions.

Discussion

The results of alternate leg lead box stepping were consistent with some E-C uncoupling even though the drop in P_t was not as large as in P_{o10} . The greater effect on P_{o10} compared to P_{o20} and the inability to sustain a steady force during 10 Hz stimulation post exercise would be expected as would the much smaller effect on MVC.

Clearly box-stepping had induced long lasting changes at low stimulus frequencies which running did not, though the workload in the two exercises was similar. A possible explanation for this was the eccentric contractions required in lowering the body to the ground when stepping off the box, involving the lengthening of an activated muscle. In order to investigate this further the subject repeated the experiment in a way that allowed the eccentric and concentric elements of the work to be studied separately. This was achieved by requiring the subject to lead with the same leg throughout the exercise. This procedure ensured that the lower leg muscles of the trailing leg perform wholly concentric work whilst the leading leg performed eccentric contractions while slowing the body as it returns from the step to the floor during each cycle.

With this model it was clearly shown that eccentric contractions produced large, long lasting effects on force generation at low tetanic stimulus frequencies. Since no maximal high frequency 50 Hz measurements were possible in this subject, direct information regarding the presence or absence of high frequency force failure could not be gathered. Taking MVC as an indicator of high frequency force shows substantial

reductions may be induced by this form of exercise though the reductions are not as great as at low frequencies. This model used to study eccentric and concentric contractions therefore appeared to warrant further study as it strongly indicated that long lasting changes particularly at low stimulus frequencies could be caused in the human triceps surae.

Box Stepping (ii)Introduction

If the mechanism responsible for the lasting effects of box stepping was E-C uncoupling then the synchronous surface recorded action potential measured during stimulation at any frequency should remain unchanged while force falls. This was in fact the case in the study of Edwards et al (1977b) on adductor pollicis when no change in shape or amplitude of the action potential was detected during tetani at 20 and 100 Hz though there was a significant loss of force at the lower frequency. In that study contractions were evoked by stimulation of the ulnar nerve at the wrist and because of nerve conduction time the stimulus artifact did not interfere with recording of the synchronous action potential from the muscle belly. If similar recordings are attempted from the belly of a muscle between two large surface stimulating electrodes as in the case of quadriceps or the triceps surae then the stimulus artifact will obscure the early phase of the muscle action potential. However, Hultmann and Sjöholm¹¹ (1983) developed a technique used on the quadriceps whereby a signal of equal and opposite amplitude and equal time course to the artifact was generated by an electrical circuit. With careful adjustment this signal could then be used to cancel out the stimulus artifact and leave the synchronous muscle action potential intact. By using supramaximal stimulation to evoke a maximal response while monitoring the synchronous muscle action potential it would be possible to identify uncoupling of

excitation and contraction in the human triceps surae. These experiments, aimed to systematically investigate the effects of constant leg lead box stepping on the contractile characteristics of the triceps surae with particular reference to the reasons underlying the long lasting force loss following eccentric contractions. In these experiments subjects were used only if they could tolerate supramaximal 50 Hz stimulation on both legs, allowing direct comparison of maximal responses at high and low frequency.

Methods

Subjects for these experiments were five men, mean age 21.4 ± 3.8 years, height 176.6 ± 6.2 cm and weight 71.1 ± 6.5 kg. All were habituated to the involved procedures over 4 or 5 preliminary visits to the laboratory during which they became tolerant of supramaximal stimulation at tetanic frequencies up to 50 Hz. The apparatus and stimulation technique were as described in Chapter II. On the experimental day the protocol was as follows. Twitch responses were evoked by stimulation at increasing voltage, starting with a voltage which just produced a measurable force and ending with voltages which failed to increase the evoked response. From a U-V record of the maximal response P_t , TPT and $1/2$ RT were measured. Tetanic responses at frequencies of 10, 20 and 50 Hz were recorded, again voltage was increased until no further increase in force could be obtained. Once the maximal responses had been reached single tetani at 20 Hz and 50 Hz were evoked using the highest voltage tolerated by the subject. These responses were recorded at high speed on the

U-V paper and from these records maximum relaxation rates at 20 Hz (RF20) and 50 Hz (RF50) were measured by drawing a tangent to the steepest portion of the curve. Maximum rate of rise of the 50 Hz tetanus (RR50) was also measured in the same way, however the rate of rise of the 20 Hz tetanus could not be measured as the contraction was only partially fused at this frequency making it impossible to draw a tangent to the force record. A 2 min rest period was given before and between these two tetanic contractions. Following a further 2 min rest period three maximal voluntary contractions were recorded and after another 2 min rest period the fatigue test was performed. Here again high paper speeds were used at the start and finish of the test to allow measurement of relaxation rates of the first (RF1) and last (RF120) tetani. This protocol was carried out on both legs prior to the exercise period, which consisted of 1 hour of box-stepping with a constant leading leg, at a rate of 20 lifts of the body per minute. Constant stepping rate was ensured by use of a metronome set to 80 beats/min, each beat corresponding to one foot fall. Step height was adjusted to just below patellar height for each subject.

Immediately post-exercise the concentric leg was measured first followed by the eccentric leg. Two hours after exercise ended the concentric leg was re-measured followed by the eccentric leg and this was repeated 24 hours after the end of the exercise. EMG recordings were made from the belly of the soleus as described in Chapter IV section 2. Signals were displayed on an oscilloscope and recorded on FM Tape (Racal).

Results

Twitch responses are summarised in Table 5.4. In the concentric leg P_t was significantly reduced by 11% after exercise and had recovered after 2 hours, $1/2$ RT was also significantly reduced following exercise but had again recovered after 2 hours. TPT was not significantly affected by the exercise.

The eccentric leg showed a mean fall in P_t of 22%, double that of the concentric leg, and was still significantly reduced by an average of 11%, two and a half hours post-exercise. Recovery was complete after 24 hours. TPT was significantly reduced immediately after exercise but had recovered after two and a half hours, while $1/2$ RT was unaffected by the exercise.

Table 5.5 shows the mean values for the maximal evoked tetanic and voluntary contractions of the concentric leg. One subject was unable to tolerate supramaximal tetanic stimulation or perform an MVC on his concentric limb on the experimental day because of a prior thigh injury directly beneath the site of the force plate. For this reason the force values in Table 5.5 are the mean of four subjects. In a second subject a pronounced reflex always occurred during relaxation after the 50 Hz tetanus in one leg making it impossible to measure accurately the maximum relaxation rate. This limb became the concentric leg as the major interest in these experiments was the eccentric leg, consequently the value in Table 5.5 for RF50 is the mean of three subjects. The only significant effects of stepping on tetanic force generation in the concentric limb were a reduction in P_{010} and P_{020} both by an average of 13%, the slight falls in mean P_{050} and MVC were not significant. The fatigue test showed

TABLE 5.4.

The effects of box stepping, with constant leg lead, on the twitch characteristics of the triceps surae performing eccentric and concentric contraction.

<u>ECCENTRIC LEG</u>			N=5	<u>CONCENTRIC LEG</u>		
TPT (msec)	1/2RT (msec)	Pt (N)		TPT (msec)	1/2 RT (msec)	Pt (N)
Control	126 ±12.3	87 ±6.1	118 ±20.3	128 ±12.8	91 ±8	128 ±25.3
Post-Ex	109* ±15.6	86 ±10.4	92*** ±24.9	120 ± 8.1	81* ±4.8	114* ±31.4
Recovery	115 ±14.8	85 ±6.2	105* ±21.9	125 ±15.1	85 ±3.1	132 ±32.3
+24 Hrs	115 ±18.4	89 ±10.5	119 ±19.1	123 ±15.8	94 ±6.9	126 ±31.0
*** 0.01>p>0.001				* 0.05>p>0.02		

TABLE 5.5.

The effects of constant leg lead box stepping on maximal tetanic and voluntary force, fatiguability and rate of rise and fall of force in the concentric leg. (n = 4)

	FORCES				RATES						
	P _O 10	P _O 20	P _O 50	MVC	FI	RF ₂₀	RF ₅₀ [†]	RF ₁	RF ₁₂₀	FI _R	RR ₅₀
	(N)	(N)	(N)	(N)		%Pomsec ⁻¹	%Pomsec ⁻¹	%Pomsec ⁻¹	%Pomsec ⁻¹		%Pomsec ⁻¹
Control	604 ±97	850 ±107	1062 ±127	1353 ±169	0.70 ±0.09	1.73 ±0.29	1.31 ±0.19	1.14 ±0.20	0.77 ±0.17	0.68 ±0.08	0.32 ±0.05
Post-Ex	525* ±130	735* ±162	987 ±193	1257 ±242	0.69 ±0.09	1.92 ±0.20	1.35 ±0.11	1.27* ±0.15	0.85 ±0.23	0.67 ±0.15	0.34 ±0.05
Recovery	557 ±147	810 ±187	1041 ±182	1344 ±181	0.69 ±0.07	1.81 ±0.38	1.43 ±0.18	1.12 ±0.17	0.78 ±0.16	0.70 ±0.14	0.32 ±0.04
+24 Hrs	599 ±129	829 ±172	1029 ±203	1236 ±240	0.70 ±0.06	1.75 ±0.27	1.44 ±0.25	1.08 ±0.07	0.73 ±0.15	0.68 ±0.12	0.31 ±0.07

*
0.05>p>0.02

† N = 3

no significant change in initial force or force loss over the 2 minutes of intermittent stimulation, therefore the FI remained constant. However the mean relaxation rate of the first tetanus (RF1) was significantly increased following exercise while the slight increase in mean relaxation rate of the 120th tetanus (RF120) was not significant, the ratio of the two relaxation rates FI R was not significantly changed by concentric exercise.

Stimulus response curves for both 20 and 50 Hz tetanic stimulation showed enhanced responses at submaximal voltages post-exercise (Fig. 5.7a and 5.7b) and a slight reduction in maximal P_{020} can also be seen (Fig. 5.7a) post-exercise.

The eccentric limb responses to supramaximal tetanic stimulation and maximal voluntary effort are summarised in Table 5.6. There were significant and substantial falls in tetanic tension at all stimulus frequencies post-exercise which had not fully recovered after 24 hours. The relative falls in 10, 20 and 50 Hz responses post-exercise were 54, 36 and 15% respectively indicating a progressively smaller effect of eccentric exercise on tetanic force generation with increasing stimulus frequency. The 10 Hz response 'sagged' (see Burke et al, 1976) post-exercise in all subjects.

The frequency difference was still marked after 24 hours recovery when the respective 10, 20 and 50 Hz forces were reduced by 24, 18 and 10% but clearly the lower frequency forces had recovered relatively more than the high.

The typical stimulus response curves for 20 and 50 Hz stimulation of the eccentric leg (Figures 5.8a and 5.8b) show enhancement at the lowest voltages and reduction at higher

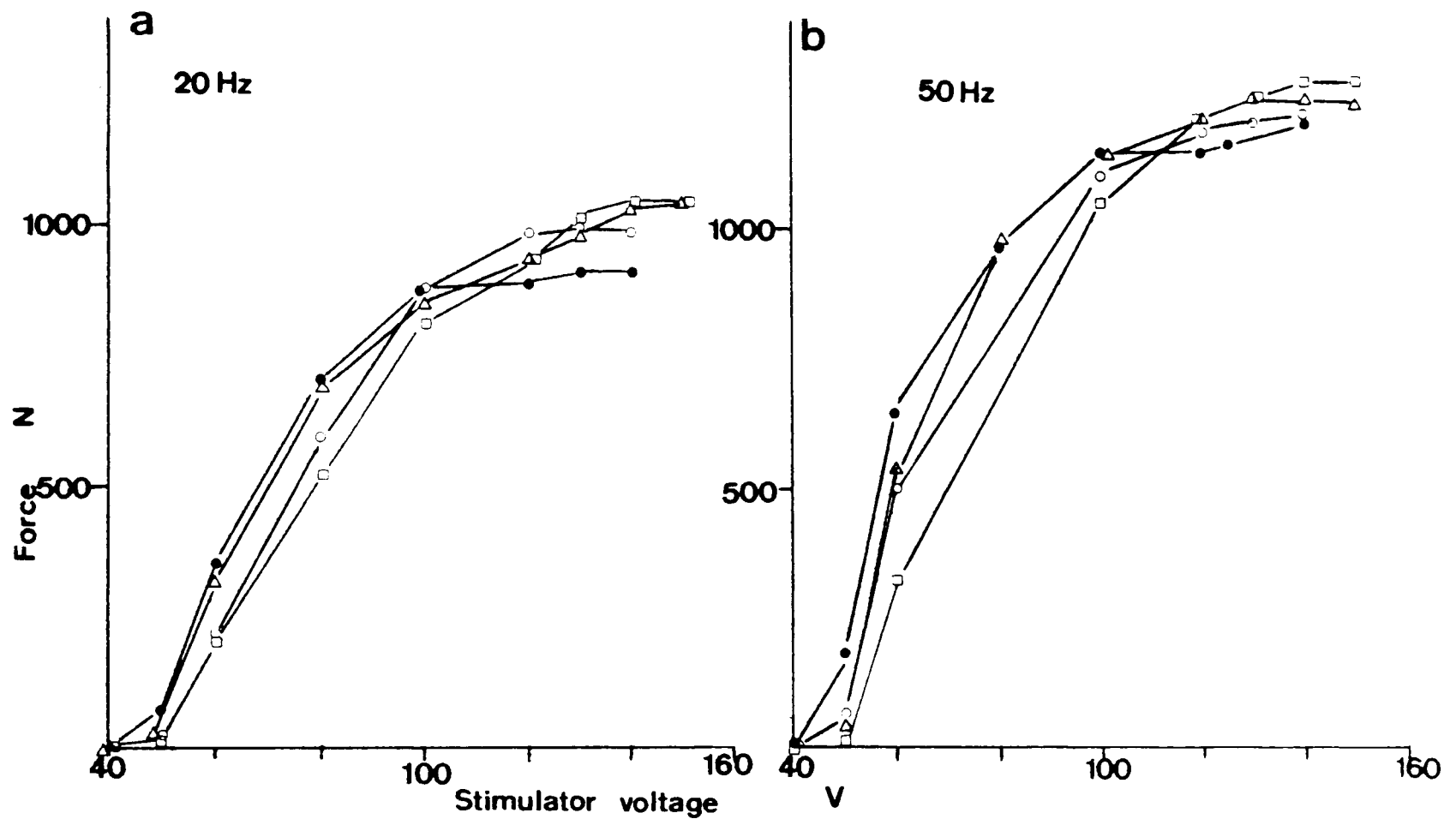


Figure 5.7.

Typical 20 and 50 Hz stimulus response curves from the concentric leg before, after and during recovery from 1 hour of constant leg lead box stepping.

Symbols; (o) control, (●) post-exercise, (Δ) recovery and (□) after 24 hours.

TABLE 5.6.

The effects of constant leg lead box stepping on maximal tetanic and voluntary force, fatiguability and rate of rise and fall of force in the eccentric leg. (n=5)

	FORCES				RATES						
	P _O 10	P _O 20	P _O 50	MVC	FI	RF ₂₀	RF ₅₀	RF ₁	RF ₁₂₀	FI _R	RR ₅₀
	(N)	(N)	(N)	(N)		%Pomsec ⁻¹	% Pomsec ⁻¹	%Pomsec ⁻¹	%Pomsec ⁻¹		%Pomsec ⁻¹
Control	685 ± 168	960 ± 215	1179 ± 240	1580 ± 324	0.66 ± 0.15	1.87 ± 0.22	1.52 ± 0.17	1.17 ± 0.09	0.81 ± 0.22	0.69 ± 0.17	0.37 ± 0.03
Post-Ex	318*** ± 188	610*** ± 275	1008** ± 285	1338*** ± 279	0.83** ± 0.10	1.75 ± 0.58	1.73 ± 0.21	1.27* ± 0.25	0.98 ± 0.20	0.77 ± 0.07	0.36 ± 0.02
Recovery	396*** ± 141	638*** ± 219	996*** ± 233	1384* ± 276	0.79* ± 0.07	1.51* ± 0.27	1.65 ± 0.10	1.11 ± 0.08	0.90 ± 0.12	0.81 ± 0.07	0.38 ± 0.03
+24 Hrs	524* ± 107	787* ± 169	1065* ± 189	1447 ± 305	0.74*** ± 0.13	1.58* ± 0.27	1.50 ± 0.26	1.09 ± 0.13	0.84 ± 0.18	0.76 ± 0.12	0.36 ± 0.06

* 0.05>p>0.02 ** 0.02>p>0.01 *** 0.01>p>0.001

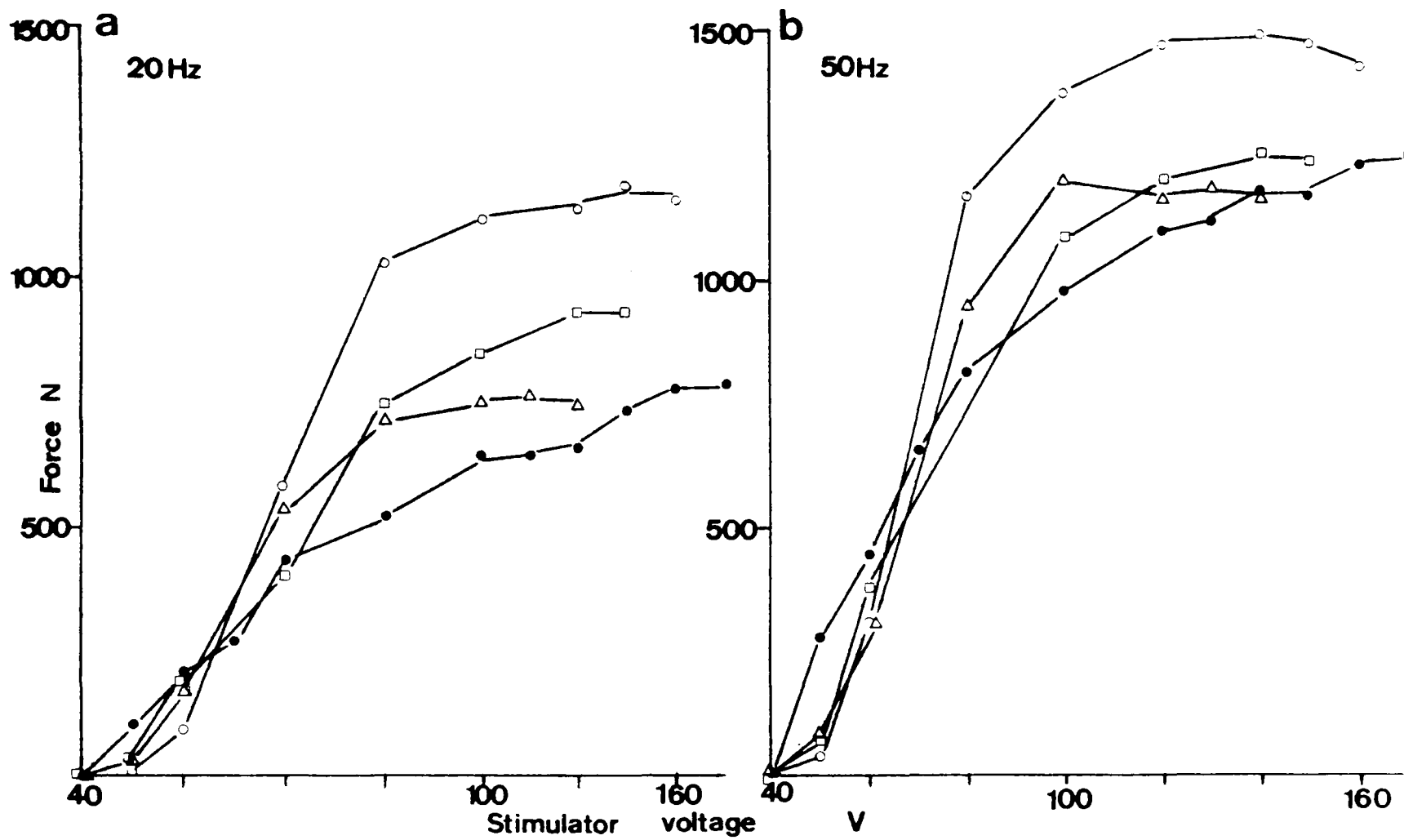


Figure 5.8.

Typical 20 and 50 Hz stimulus response curves from the eccentric leg before, after and during recovery from 1 hour of constant leg lead box stepping.

Symbols; (o) control, (●) post-exercise, (Δ) recovery and (□) after 24 hours.

voltages following exercise. Gradual recovery of the 20 Hz response can also be seen.

Voltage dependence of the 20:50 ratio could be demonstrated in both concentric and eccentric limbs (Fig. 5.9), though the effect was not so marked in the eccentric limb following exercise and during recovery. The mean maximal 20:50 ratio in the eccentric leg fell from 0.81 ± 0.03 to 0.58 ± 0.11 ($p < 0.02$) after exercise recovering to 0.73 ± 0.04 after 24 hours though this was still a significant reduction from control ($p < 0.05$). The ratio was also reduced in the concentric leg post-exercise from the control value of 0.80 ± 0.02 to 0.74 ± 0.03 ($p < 0.05$) but had recovered within two hours.

MVC was significantly reduced by 15% following eccentric exercise and remained so two and a half hours later when still 12% below control, though after 24 hours recovery it was not significantly different from control.

The fatigue index (FI) of the eccentric leg was significantly increased after exercise and for the 24 hours following because of a significantly reduced ($p < 0.05$) force evoked at the start of the test. Force of the final, 120th contraction of the test was not significantly ($p > 0.05$) different from control at any time following exercise or during recovery. The slight increases in mean RFl and RFl20 post-exercise were not significantly different from control neither was the rise in FI R. Comparison of the two fatigue indices FI and FI R (Fig. 5.10), showed a significant association ($p < 0.001$; $r = 0.66$) with points scattered around the line of unity. This association was unaffected by either concentric or eccentric exercise.

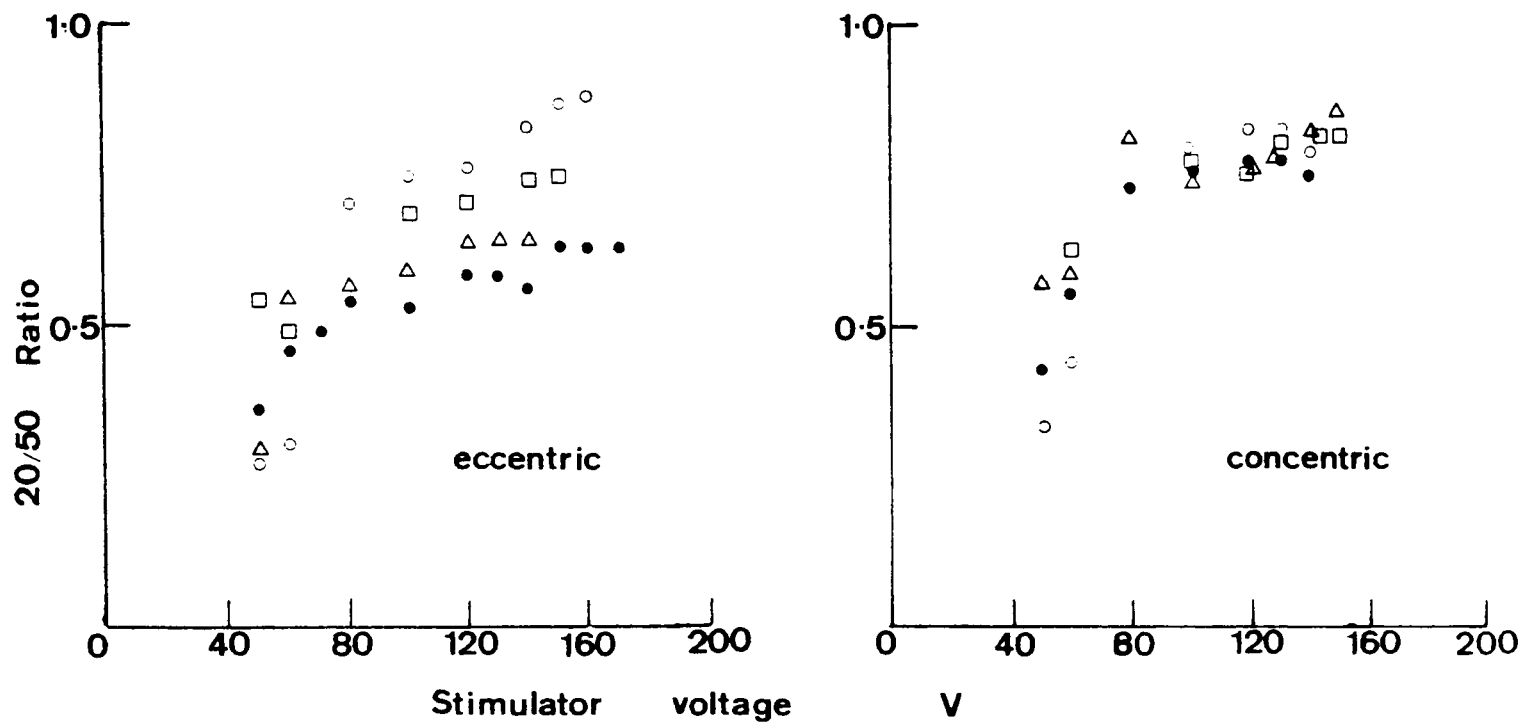


Figure 5.9.

The effects of box stepping for 1 hour with constant leg lead on the voltage dependence of the 20/50 ratio. Typical responses from the eccentric and concentric legs of a single subject are shown.

Symbols; (o) control, (●) post-exercise, (Δ) recovery and (□) after 24 hours.

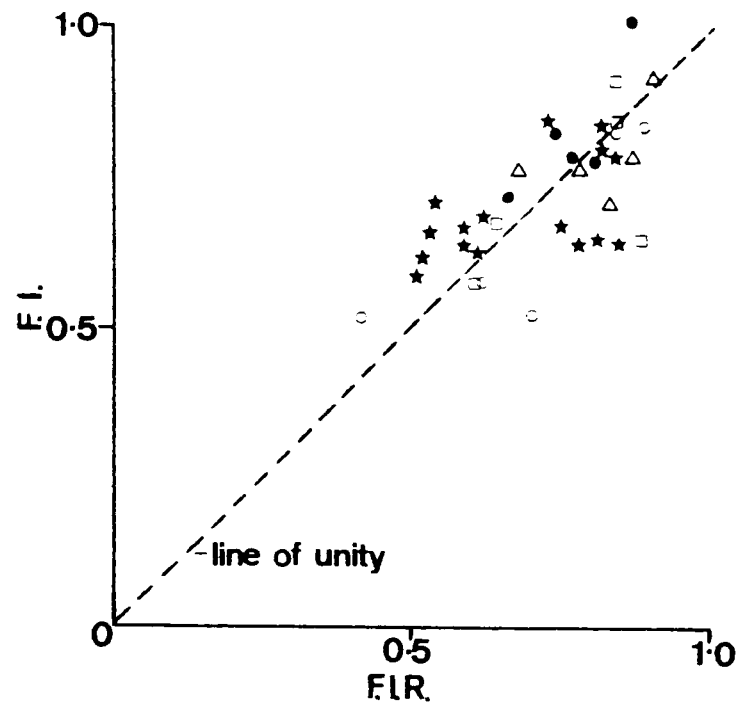


Figure 5.10.

The effects of constant leg lead box stepping on the relationship between fatigue indices calculated from force, F.I. and relaxation rate, F.I.R. in concentric (*) (n=4) and eccentric legs (n=5).

Symbols; (o) control, (●) post-exercise, (Δ) recovery and (□) after 24 hours.

In the eccentric leg mean RF20 was slightly, though not significantly reduced post-exercise but was significantly reduced after two and a half hours recovery and was still significantly lower than control after 24 hours recovery. RF50 was not significantly affected during this time but in contrast to RF20 showed a tendency to increase post-exercise. No significant change in RR50 was seen either post-exercise or during recovery. A strong inverse relationship ($r = -0.83$; $p < 0.001$) was found between TPT and RR50 when all the control data from both legs was used described by the equation:

$$\text{TPT (msec)} = - 271 \times \text{RR50 (\% Po msec}^{-1}) + 218$$

Fig. 5.11 shows the relationship between TPT and RR50 for both legs before and after exercise.

Electromyograms

The muscle action potential (MAP) was recorded in the eccentric leg of only two subjects under control and post-exercise conditions. It was found that the adjustments of the tuned circuit used to cancel the stimulus artifact were very time consuming and it was felt that the delay in making these EMG recordings would prejudice other measurements especially post-exercise.

Figure 5.12, shows typical records of the last MAP of the 10 Hz train followed by the first two MAP's of the 20 Hz train evoked by supramaximal stimulation of the eccentric leg post-exercise. In Figure 5.13, the transition from 20 Hz tetanus to 50 Hz tetanus is shown in the same stimulation train and Fig. 5.14, shows the last MAP's of the 50 Hz stimulation. The

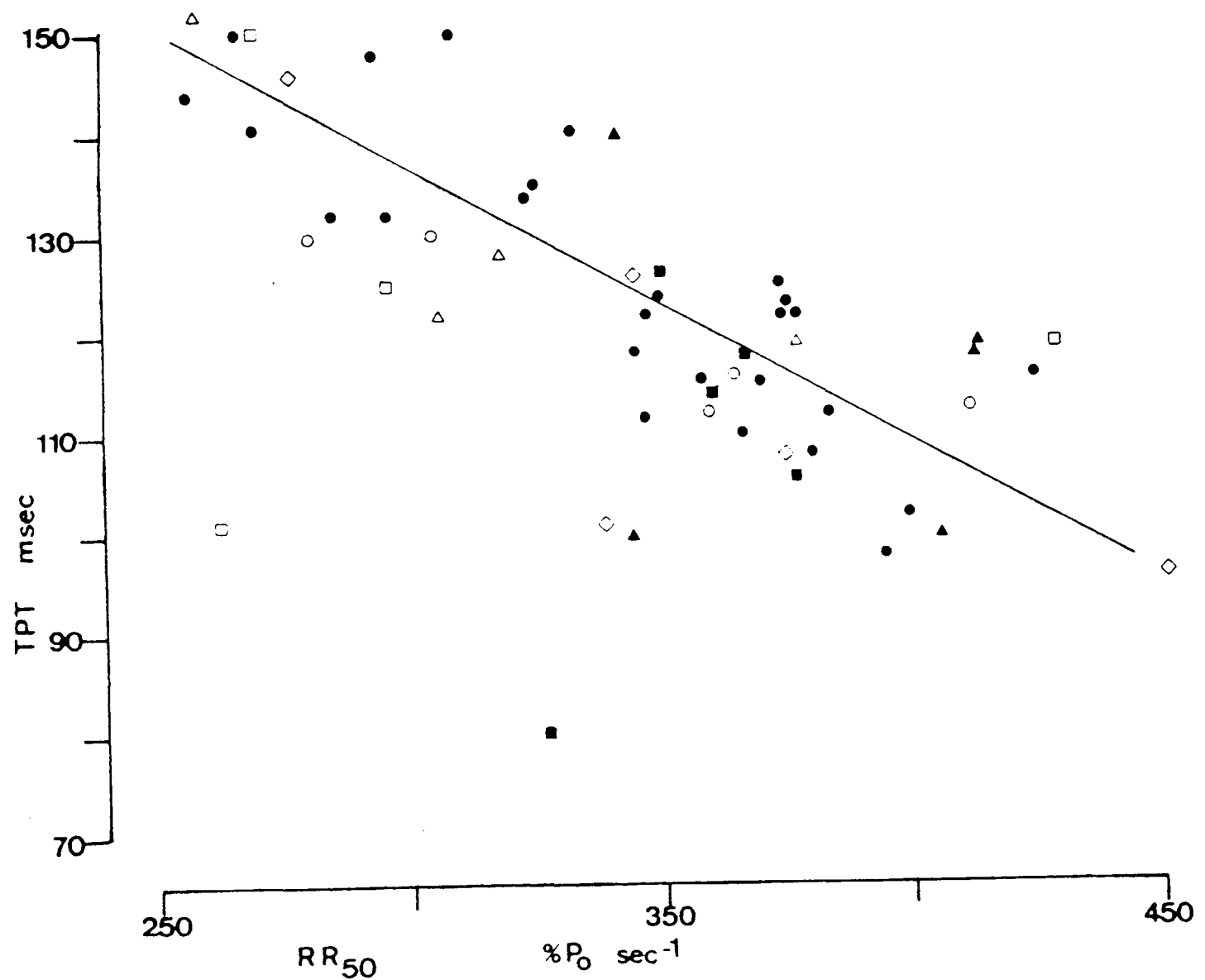


Figure 5.11.

The relationship between twitch time to peak tension, TPT, and the maximum rate of rise of a 50 Hz tetanus, RR_{50} , in 5 young men before and after constant leg lead box stepping. Control data from both legs, (●) are described by the equation $TPT \text{ (msec)} = -271 RR_{50} + 218$ ($r = -0.83$) as indicated by the solid line.

Values for the concentric leg post-exercise (○), recovery (△), plus 24 hours (□) and eccentric leg post-exercise (■), recovery (▲), plus 24 hours (◇) are shown.

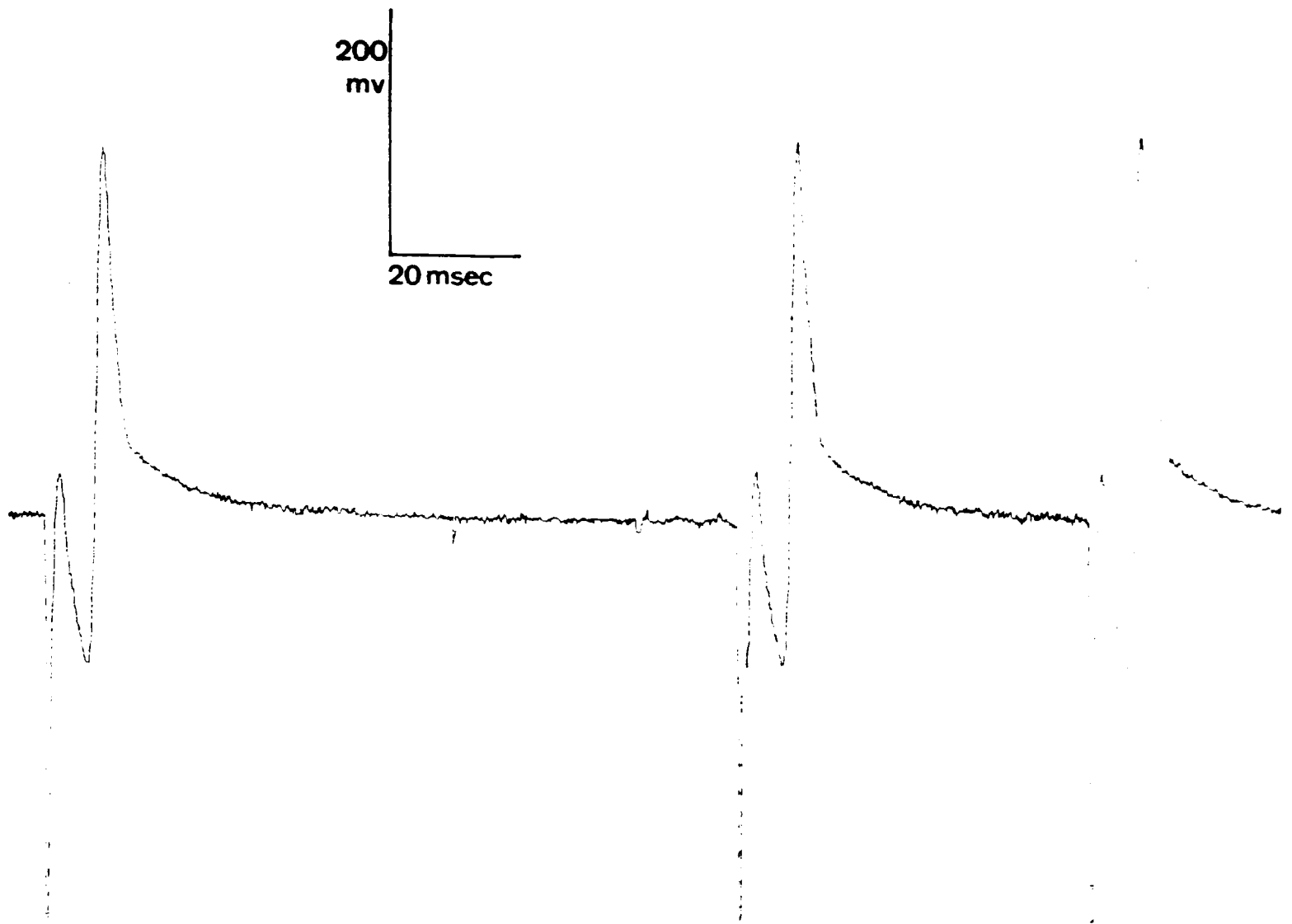


Figure 5.12.

Typical records of synchronous muscle action potentials recorded during the transition from 10 Hz to 20 Hz stimulation in the eccentric leg of one subject post-exercise.

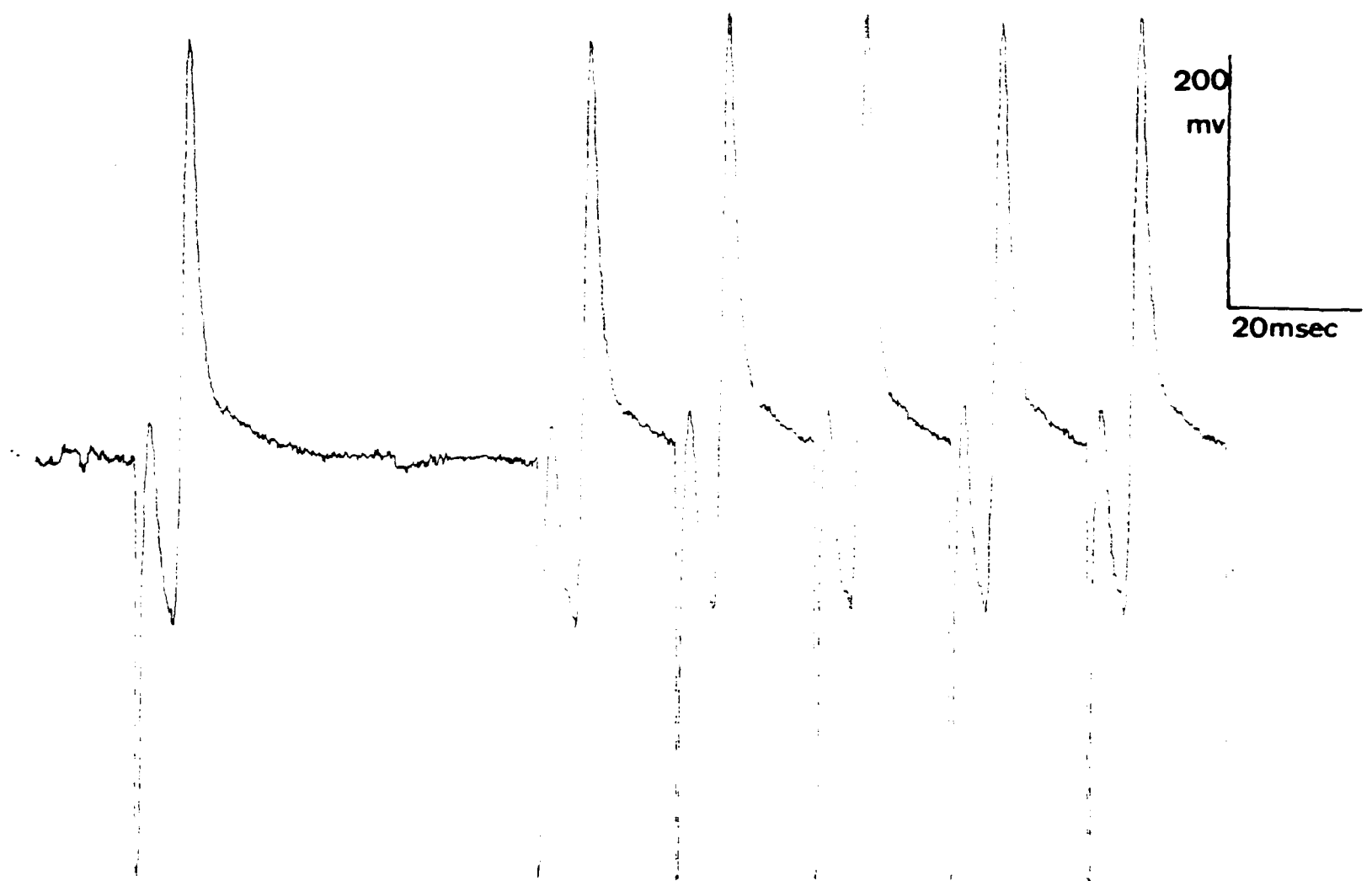


Figure 5.13.

Synchronous muscle action potentials recorded during the transition from 20 to 50 Hz stimulation in the same stimulus train as seen in Fig. 5.12.

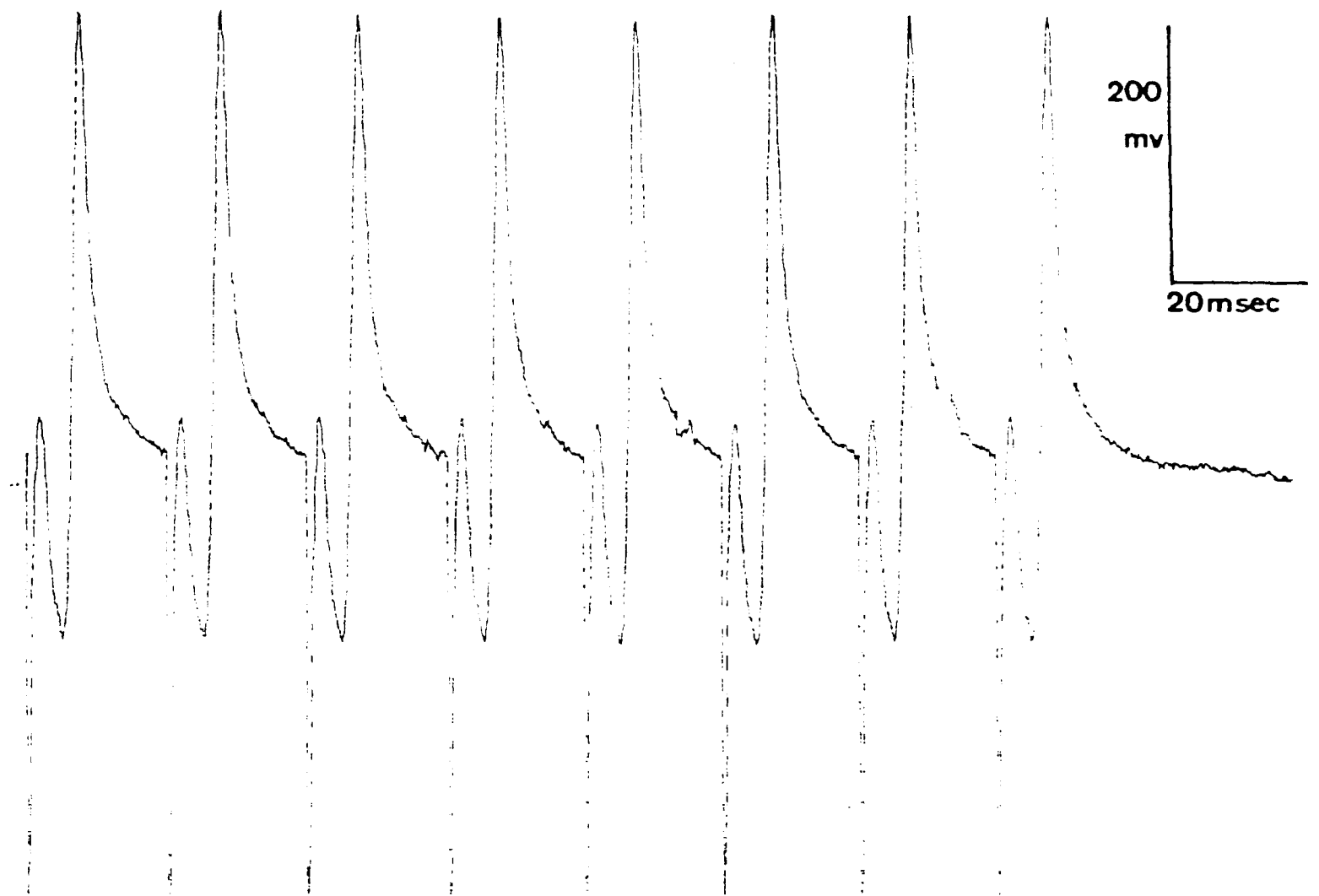


Figure 5.14.

The last synchronous muscle action potentials of the 50 Hz stimulus train begun in Fig. 5.13.

MAP's showed a slight tendency to grow in amplitude during a tetanic train but this also occurred during control stimulation and is probably due to a breakdown of leg resistance during stimulation. The time course of the MAP's measured peak to trough, remained almost unchanged during the tetani. Clearly failure to excite the muscle cannot account for the reduction in P_{O10} to 49% of control and P_{O20} to 68% control which were the values at the time of recording.

Discussion

The results of these box-stepping experiments clearly confirmed those of the pilot study which indicated that large and lasting changes could be induced in the contractile characteristics of the triceps surae when forced to perform repeated eccentric contractions.

When interpreting the results of supramaximal stimulation of whole human muscles it must be remembered that the force seen at the transducer is the resultant of all the forces of the different muscle fibre types within the stimulated muscles. Changes in twitch amplitude and time course for example may therefore be due to a change in the force contribution of a proportion of the muscle fibres (Biscoe and Taylor, 1967) or to a more general change in all the fibres. Twitches evoked from the concentric leg post-exercise showed a significant fall in P_t and $1/2 RT$. It is tempting to ascribe both changes to a raised muscle temperature as the measurements were made not more than 10 min after the end of exercise and the effects were short lasting. However, a fall in P_t was not seen after passive

heating of the muscle or active heating nor after one hour running when a similar muscle temperature must have been reached. Under these latter conditions a faster twitch which developed a normal force would indicate a faster rate of rise of force. This would be explained in terms of the classical model of contraction if, though the duration of the active state were reduced the shortening velocity of the contractile component was increased by the raised muscle temperature allowing greater force generation in a shorter time (Hill, 1951; Simmons and Jewell, 1974; Julian and Moss, 1976). Since activation is governed by calcium release and reuptake (Endo, 1977) and velocity of shortening (V_{max}) related to myosin ATPase activity (Bárány, 1967) it is possible that activity of both enzyme systems could be increased by temperature in such a way that P_t is kept constant (Hill, 1951). In the present experiments then, the reduced P_t of the concentric leg post-exercise could be due to a more rapid deactivation process combined with a proportionately smaller increase in shortening velocity of the contractile component. More rapid deactivation could also account for the decreased $1/2 RT$ and the slight fall in mean TPT (Fig. 5.15). Alternatively P_t could be lower due to a decrease in the amount of calcium released in response to a single stimulus (Edwards et al, 1977b). This model, assuming calcium re-uptake progresses at the normal rate, results in the removal of calcium from the contractile proteins in a shorter period of time. P_t and TPT would be reduced because of this briefer 'active state' but twitch relaxation rate, $1/2 RT$, would not be affected.

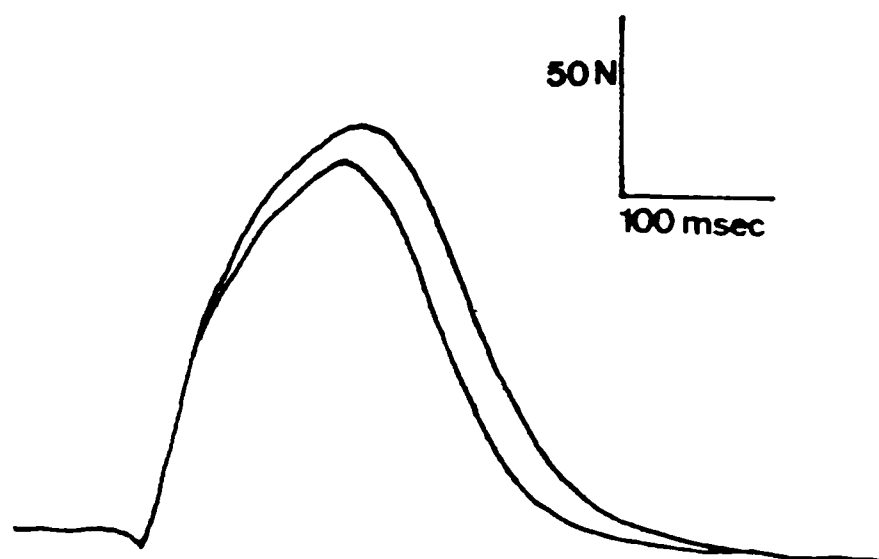


Figure 5.15.

Tracings of original U.V. records showing maximal twitch responses before and after box stepping in the concentric leg. The post-exercise response is slightly smaller and quicker. Subject M.W.

A further possible explanation of the changes seen in P_t , TPT and $1/2$ RT is complete loss of force produced by the slowest contracting fibres. This would reduce the resultant twitch force and make it appear faster. However, if the loss of force from these slow fibres was anything other than complete the total time course of the resultant twitch would remain unaltered.

The reduction in P_t in the eccentric leg was double that of the concentric leg post-exercise and there was also a marked fall in TPT. P_t had not recovered after two and a half hours but mean TPT though reduced was not significantly different from control at this time. A reduction in force contributed by slower fibres would, as already described, possibly reduce TPT and P_t , however it would also tend to increase $1/2$ RT as total contraction time must be maintained and this was not seen in these experiments. Muscle temperature must have fallen considerably by the time the post-exercise measurements were made (+20-25 min) and after two and a half hours would be expected to be normal, therefore its effect may be discounted on these grounds alone. In addition the finding of a normal $1/2$ RT post-exercise would not be expected if muscle temperature were still raised. These changes in the twitch are probably best explained by a shorter duration of active state caused by diminished Ca^{2+} release. This is the mechanism proposed for low frequency fatigue (Edwards et al, 1977b; Grabowski et al, 1972). A reduction in the amount of Ca^{2+} released by a single stimulus need not affect initial rise of tension, however its reuptake would require a shorter period of time, consequently

deactivation would occur at an earlier stage resulting in reduced P_t and TPT (Fig. 5.16). The finding of a constant $1/2$ RT would also be compatible with this explanation.

Tetanic stimulation of the concentric leg post-exercise showed small but significant falls in P_{010} and P_{020} but not at P_{050} indicating a shift of stimulation response curve to the right. This would be expected of a faster muscle with a more rapid rate of deactivation requiring a high stimulus rate to maintain full activation. The significant increase in RF1 and the raised mean values for RF120 and RF20 all would support this as does the significant decrease in twitch $1/2$ RT. Raised muscle temperature is the most likely explanation of these changes but it must be remembered that while passive heating reduced P_{010} by a mean of 15% it had no effect on P_{020} , here P_{010} and P_{020} were both reduced by a mean of 13%.

A second method by which loss of force at low frequencies relative to high could be produced is by a reduction in force contribution by the slowest muscle fibres which produce fully fused responses at low frequencies of stimulation (Cooper and Eccles, 1930; Buller and Lewis, 1965; Garnett et al, 1979). These fibres contribute most to the force evoked by low frequency whole muscle stimulation, as faster fibres produce unfused weaker contractions at such low frequencies. One might then expect a similar absolute loss of force from the whole muscle at all stimulus frequencies above the fusion frequency of these slow fibres because their stimulus response curve flattens above this frequency. The mean losses of force after exercise at 10, 20 and 50 Hz were 79N, 115N and 75N respectively lending

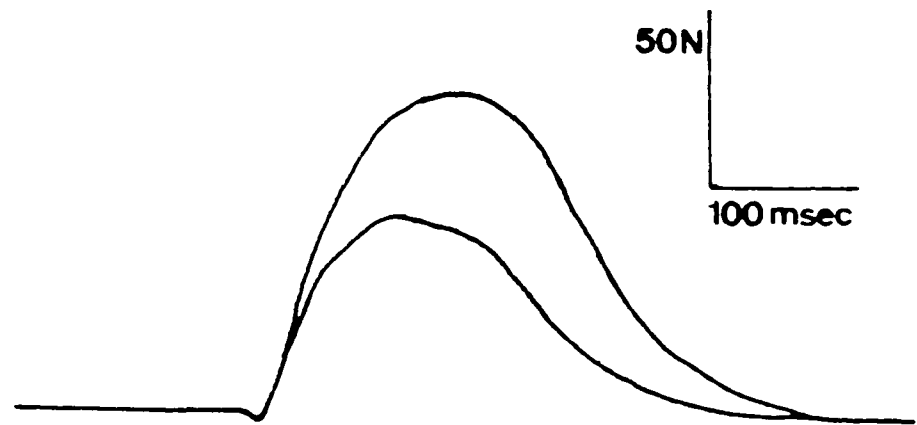


Figure 5.16.

Tracings of original U.V. records of maximal twitch responses before and after box-stepping in the eccentric leg. Post exercise the twitch is markedly smaller with a shorter TPT but normal $\frac{1}{2}$ RT.

Subject C.W.

some support to this hypothesis though the effect is very small and the drop in P_{050} was not significant. However as already discussed the twitch data does not support this hypothesis because the total twitch time would not be affected by a slight loss of force from slower fibres and in fact it was (Fig. 5.15).

A third explanation for the results is the low frequency fatigue effect (Edwards et al, 1977b). This should become progressively less noticable with increasing frequency of stimulation and while the 20:50 ratio was significantly reduced post exercise the relative reduction in P_{010} and P_{020} were equal. Also the twitch changes seen may not be compatible with the phenomenon as already discussed.

In the eccentric limb the changes in tetanic and voluntary forces were clear cut and lasting. Post-exercise tetanic force was reduced at all frequencies but the loss of force became relatively less with increasing frequency. Failure of excitation at low frequency could not account for this result as shown in Figures 5.12; 5.13 and 5.14 when the MAP recorded during 10, 20 and 50 Hz stimulation was shown to be normal. These results are as would be expected if some uncoupling of excitation and contraction had occurred (Edwards et al, 1977b) a decrease in the amount of activation per pulse being less debilitating at higher frequency where the total level of activation would be less affected. Recovery of force was slow and not complete at any frequency after 24 hours when the experiments ended. Low frequency forces had recovered relatively more quickly at this stage, P_{010} having improved on its original force deficit by 56%, P_{020} by 50% and P_{050} by only

33% but the differential effect of frequency was still present. Edwards et al (1977b) found a 90% recovery of 80 Hz force within 30 min of the end of exercise in adductor pollicis but they could not say with certainty whether there was a slow phase of recovery of force at this high frequency of stimulation because of the variability in maximum force of between 5-10% during the 24 hours of measurement. In the present study no measurement of tetanic force was made until some 30 min after eccentric exercise so the rapid recovery phase may have been missed but certainly evidence exists here supporting a slow recovery phase at 50 Hz which is incomplete after 24 hours. The recovery of MVC after 24 hours shows that the triceps surae are capable of generating normal force at this stage and no lasting damage to the contractile apparatus had been sustained. However, all the subjects complained of soreness in the eccentrically exercised muscles of calf and thigh, which was first noticed on the day after exercise becoming worse on the second day then gradually subsiding over the next 3-5 days (Newham et al, 1983). Substantial damage to muscle can be caused by eccentric contractions, for example McCully and Faulkner (1985) showed in mouse muscle following eccentric but not concentric or isometric contractions that 37% of the muscle fibres of extensor digitorum longus had degenerated three days after eccentric exercise. A further implication of the recovery of MVC within 24 hours, when forces evoked by stimulation at frequencies up to 50 Hz were still significantly reduced, is that firing frequencies of over 50 sec are involved in a maximal voluntary contraction of the triceps surae. Only a few studies have reported firing rates

during maximal voluntary contractions because of the inherent difficulties of making recordings from single units when many surrounding units are also active (Freund 1983). Rates of between 80 and 120 sec have been reported during maximal contractions of tibialis anterior (Desmedt and Godaux, 1977); abductor digiti minimi (Tanji and Kato, 1973; Gillies, 1973) and adductor pollicis (Marsden et al, 1971).

The relaxation rate changes seen following eccentric exercise are of great interest. Mean RF20 showed a decrease post-exercise which became significant after two and a half hours of recovery and was still present after 24 hours. In contrast, during this period RF50 showed no significant change tending to increase post-exercise if anything. The rate limiting step in muscle relaxation is thought to be either calcium accumulation by the sarcoplasmic reticulum (S.R.) (Sandow 1965 and 1970; Dawson et al, 1980) or cross bridge dissociation involving ATP splitting by myosin ATPase (Edwards et al, 1975). It seems unlikely that the latter fundamental property of the muscle contractile apparatus could be changed at one stimulus frequency, 20 Hz, but not at the other, 50 Hz. On the other hand as the twitch and low frequency tetanic responses especially, may have been affected by changes in Ca^{2+} release from the S.R. the suggestion that Ca^{2+} reuptake by the S.R. may also be affected at low frequencies of stimulation is not untenable. If this is so, then the higher stimulus rate of 50 Hz must cause a restoration of normal S.R. efficiency as shown by the normal RR50 and RF50. Hill and Jones (1978), showed that prolonged twitches with slowed relaxation, found during recovery

from repeated tetanic trains in adductor pollicis, instantly reverted to normal following a short tetanus. Furthermore, Jami et al (1983) showed that single motor units of cat peroneus tertius with 'delayed fatigue' in response to 20-40 Hz stimulation could be made to develop nearly normal tension by gradual build up upon stimulation at 30-40 Hz. These results may indicate that both the deactivation process, in the case of Hill and Jones (1978) and the activation process, in the case of Jami et al (1983) can be forced to behave normally by an increased rate or duration of excitation. The present results would support that hypothesis. If this interpretation of the results is correct and not only is activation at low frequency depressed but deactivation also, then the latter may to some extent compensate for the former and help maintain some level of activation.

Alternatively, the reduced relaxation rate after a 20 Hz tetanus but not after a 50 Hz tetanus could indicate that the force produced at 20 Hz came predominantly from slow fibres which then dominated the shape of the relaxation curve of the whole muscle (Wiles et al, 1979). If low frequency fatigue was limited to the faster fibres (Kugelberg and Lindegren, 1979; Jami et al, 1983) then they would contribute little to low frequency tetanic contractions due to a combination of a normally high tetanic fusion frequency and a failure of activation at low frequency because of the exercise. Thus slow fibres with low fusion frequency would be the major force producers at 20 Hz following exercise, especially as they predominate in the triceps surae (Johnson, 1973; Edgerton,

1975; Gollnick, 1974c). If this were so then by the same arguments the 10 Hz response must also have been attributable primarily to slow fibres. However at this frequency the post-exercise response showed a distinct tendency to 'sag' in all subjects, that is the force developed in the early part of the tetanus was not well maintained, collapsing almost to zero in some subjects. Such a response would not be expected of normal slow muscle at this frequency of stimulation (Burke, 1976; Cooper and Eccles, 1930) and may indicate that slow fibres were affected by a failure of activation during low frequency tetani to such an extent that force generation could not be sustained. This sag at 10 Hz was not seen during the recovery measurements or after 24 hours when the statistically significant fall in RF20 was present so it may be that the slower relaxation rate at this time, was due to a predominantly slow fibre involvement as already discussed. The possibility still remains however that slow fibres affected by low frequency fatigue immediately post-exercise had recovered sufficiently to avoid sag but may not have been fully recovered. It is difficult to believe that low frequency fatigue was confined to the faster fibres because of one further factor, that is the recruitment order of fibres believed to occur in dynamic exercise. Fast fibres have higher force thresholds for recruitment than slow even in ballistic contractions when the force threshold for all types of motor units decreases as the rate of rise of tension increases (Desmedt and Godaux, 1977). Certainly with the predominance of slow fibres in the triceps surae and the orderly recruitment of fibres starting with the slowest, the involvement of faster

fibres must have been small compared to that of the slower fibres in this exercise.

The finding of low frequency 'fatigue' in the eccentric leg did not cause the muscle to be more fatiguable as judged by its response to repeated evoked contractions in the fatigue test. The relative loss of force during the 2 min test was significantly less following exercise and during recovery than under control conditions as indicated by a raised FI. This was supported by an increase in mean FIR though this was not significant. It must be remembered however, that the force evoked by the 20 Hz stimulation was significantly reduced at the beginning of the test and therefore the total force time integral of the test would be much reduced with a correspondingly lower metabolic demand on the muscle. Interestingly, the force evoked by the final, 120th, train of the fatigue test was statistically indistinguishable from control at any time following exercise or during recovery. This may complement the findings discussed earlier of Jami et al (1983) who found that nearly normal forces could be gradually developed by motor units with delayed fatigue, if given prolonged stimulation at low frequency.

The findings of a voltage dependent 20:50 ratio in both concentric and eccentric legs pre- and at all stages post-exercise, (Figure 5.9) once again illustrates the problem of using submaximal voltages on the triceps surae. Presence or absence of low frequency fatigue could not be ascertained with certainty on either leg, using a low voltage, though the reliable range of voltage above which a more stable 20:50 ratio

is found appears wider after eccentric exercise. Furthermore, the effects on high frequency force would have been missed if submaximal responses were used, since no other response could be used for comparison, giving the impression that only low frequencies of stimulation were affected.

CHAPTER VI

Summary and Conclusions

Summary and Conclusions

The aim of this thesis was to assess objectively weakness and fatigue in the human triceps surae, with particular reference to the effects of disuse, age and exercise. The development of a method to evoke maximal isometric responses using supramaximal electrical stimulation enabled a systematic investigation of weight bearing muscle function to be made in terms of absolute force generation.

The data showed that supramaximal stimulation was tolerated after a period of habituation and produced reliable and reproducible responses. Under control conditions the triceps surae of young men generated large maximal tetanic forces, were found to have a mean twitch time to peak tension of 107 msec and did not fatigue readily. This was indicative of a large muscle mass with a predominance of type I, slow twitch, fatigue resistant fibres.

Manipulation of muscle temperature over the range 39.1 to 29.5°C was without effect on maximal tetanic and voluntary responses while further cooling to 24.3°C caused a modest reduction in force generation. Twitch and unfused tetanic responses were however markedly affected by temperature. It was concluded that the temperature variations experienced by the triceps surae in normal use were unlikely to affect the capacity of the muscle to generate force. However, the processes governing activation and deactivation of contraction were highly temperature sensitive. Absolute forces generated in response to single stimuli and unfused tetani must therefore be related to the muscle temperature at which measurements are made.

Long term disuse as a result of immobilisation due to injury caused a large reduction in the force generating capacity of the triceps surae. When measured during rehabilitation maximal 20 Hz tetanic force in the injured limb was reduced by an average of 46% compared to the uninjured limb while calf muscle (plus bone) cross sectional area (CSA) estimated anthropometrically was reduced by only 16%. Substantial improvements in maximal force generation were observed during the study without appreciable change in CSA. It was concluded that anthropometry gave an unreliable estimate of the degree of muscle wastage in the triceps surae caused by long term disuse. A significant decrease in the twitch TPT of the injured limb compared to the uninjured limb was noted while the total twitch contraction time was maintained. It was suggested that this was consistent with a predominant loss of type I fibre area from the triceps surae during long term immobilisation.

In contrast short term disuse due to voluntary immobilisation in a plaster cast for a total of 2 weeks resulted in a significant prolongation of twitch TPT and 1/2 RT which rapidly recovered with a return to normal use. Maximal tetanic tension was not significantly affected by the disuse period though C.S.A. decreased by 8% and maximal voluntary force was reduced by 24%, recovering within 4 days. It was suggested that the marked increase in twitch time course within the first week of immobilisation was an early adaptation to disuse and reflected a change in the control of Ca^{2+} release and reuptake by the sarcoplasmic reticulum before any loss of contractile machinery occurred. The large fall in MVC and its rapid

recovery was then seen as a result of a central rather than peripheral limitation to voluntary force generation following short term disuse.

The triceps surae of 70 year old men were found to be significantly slower contracting, weaker and yet, paradoxically, more fatiguable than those of young men. It was concluded that the slower twitch of the elderly men could not be taken as conclusive proof of a selective loss of type II fibre area, since total twitch contraction time increased. Rather it may indicate a change in Ca^{2+} kinetics in the muscle fibres remaining. The greater fatiguability of the elderly during a standardised evoked test was not explained by failure of excitation but may be related to restriction of blood supply caused by slower muscle relaxation.

Exercise for 1 hour at a running speed of 7 mph did not significantly affect maximal twitch force while submaximal responses were enhanced or depressed. Maximal tetanic and voluntary forces showed small short lasting reductions which had recovered within 90 min, submaximal evoked responses were again enhanced. Fatiguability was unaffected by the exercise period. It was concluded that these changes indicated a small loss of force generating capacity so that the triceps surae could be termed weaker but not more fatiguable after exercise. Furthermore, without the use of supramaximal stimulation no valid comparison could have been made between control and post-exercise forces due to the voltage dependence of the submaximal responses. Indeed, the ratio of forces evoked by 20 and 50 Hz tetanic stimulation, used to indicate the presence of low

frequency fatigue, was found to be voltage dependent both before and after exercise. This together with the finding of enhanced submaximal responses post-exercise raised serious doubts about the use of submaximal 20/50 ratios in the assessment of human triceps surae function.

Eccentric contractions performed during constant leg lead box stepping were found to produce large long lasting changes in the contractile characteristics of the triceps surae. It was concluded that the loss of maximal force following this form of exercise was due to excitation-contraction uncoupling. The effects of eccentric exercise on the rates of rise and fall of tetanic forces were explained in terms of release and reuptake of Ca^{2+} by the sarcoplasmic reticulum. It was concluded that Ca^{2+} kinetics are likely to be markedly affected by repeated eccentric contractions performed by a large weight bearing muscle.

In conclusion this thesis has demonstrated the vital importance of, supramaximal stimulation in the objective assessment of weakness and fatigue in the human triceps surae.

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LIST OF ABBREVIATIONSContractile Characteristics

Pt	Twitch tension
TPT	Twitch time to peak tension
1/2 RT	Twitch half relaxation time
P_{010}	10 Hz tetanic tension
P_{020}	20 Hz tetanic tension
P_{050}	50 Hz tetanic tension
MVC	maximal voluntary contraction
RF20	Rate of fall of 20 Hz tetanus
RF50	Rate of fall of 50 Hz tetanus
RF1	Rate of fall of 1st tetanus during fatigue test
RF120	Rate of fall of 120th tetanus during fatigue test
RR50	Rate of rise of 50 Hz tetanus
F.I.	Fatigue index
F.I.R.	Fatigue index of relaxation
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Ca^{2+}	Calcium ion
C.S.A.	Muscle (plus bone) cross sectioned area
E-C	Excitation - contraction
M.A.P.	muscle action potential
S.A.P.	Surface action potential
S.R.	Sarcoplasmic reticulum
S.r.e.	Smoothed rectified electromogram
Tm	Muscle temperature